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THE DEVELOPMENT OF THE CLOACA IN HUMAN EMBRYOS

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Indiana University

From the Anatomical Laboratory, Johns Hopkins University

SEVEN FIGURES

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INTRODUCTION

The active work in the embryology of the urogenital system began about 1880 and continued for a period of some fifteen years when Keibel's monograph was published. The writer's investigation of the cloacal region was started in 1903, and was undertaken in part as a control of Keibel's work, and in part with a view toward solving some of the points on which differences in opinion existed. The publication of this report has been delayed in the hope that certain facts in comparative embryology might be established and help to clarify some of the obscure relations found in the human embryo. Inasmuch as the extensive investigations of Fleischmann and his students have come to naught in this respect, the major differences in opinion will be considered,

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and the doubtful points answered in so far as it is possible. The short literature review covers the essential facts and effort has been made to reduce the description of the material to a concise tabulation. The writer expresses his indebtedness to Prof. Keibel at whose suggestion the development of the later stages in the embryology was undertaken, and to Prof. F. P. Mall for the use of his collection of embryos and for the many courtesies shown him in the Anatomical Laboratory of Johns Hopkins University.

Born's article ('93) reviews the earlier development of the cloacal region in a very complete manner, and the substance is as follows: The entoderm of the enteron comes into direct relation with the surface ectoderm in the pharyngeal and cloacal membranes during the formation of the head and tail folds. Both of these membranes lose their primitive position and become folded into the substance of the embryo through increase in the surrounding mesoderm. The allantois, which is developed dorsally in the mammalian embryo (human and guinea pig excepted), shifts to a ventral position on the gut, and is gradually displaced from its intimate relation to the yolk sac through increase in the amount of mesodermal tissue. The primitive streak is carried to the ventral surface of the body during the formation of the tail fold, and forms the whole or part of the cloacal membrane. Kölliker ('83), Strahl ('83, '84), and Bonnet ('88) believe that the caudal end of the primitive streak is made up of applied layers of ecto- and entoderm, and that it enters as such into the formation of the cloacal membrane. Keibel ('88) argues that this primitive relation of the ecto- and entoderm is lost through interposition of mesoderm; the latter disappearing later with restoration of the original two layered condition.

The model of the 4.2 mm. human embryo presented by Keibel ('88) shows the hind gut and widely lumened allantois opening cephalward into the caudal entodermal sac or cloaca. Ventrally, this cloaca is limited as far as the dermal navel by the epithelial cloacal membrane. Caudalward, the limit of the cloacal membrane comes about by a mesodermic separation of the epithelial layers. The gut segment distal to the lower limit of the cloacal membrane may be termed the tail gut and terminates in an undifferentiated cell mass formed by itself, the chorda and neural tube. Born emphasizes the length of the cloacal membrane as follows: "I call particular attention to the original extent of the cloacal membrane. It reaches cephalward to the point where the allantois leaves the body at right angles; *i.e.*, as far as the caudal border of the dermal navel."

The cloacal membrane does not extend as far as the allantois in the model of an 8.0 mm. human embryo presented by Keibel ('91). Mesodermic tissue has apparently wandered in from above and separated the layers of epithelium. The cloacal membrane is therefore shorter than in the 4.2 mm. stage. The tail gut shows degeneration. The precloacal mesodermic tissue has increased in amount but instead of displacing the cloacal membrane caudalward, has folded it into the lower surface of the genital tubercle as was first described by Tourneux ('89), and verified by Retterer ('90) and Reichel ('93). No intermediate stage in the development has been described up to this time ('93). With the increase in size of the genital tubercle, the entodermal cloaca becomes more deeply placed as was demonstrated by Reichel ('93). Born states that the epithelial plate contained within the genital tubercle is ectodermic and that it is continuous with the superficial ectoderm. The epithelial plate occupies the caudal surface of the eminence and is bordered laterally by folds of mesoderm (repli ano-genitaux of Retterer), while the cloacal membrane itself terminates at the postanal fold (replis postanal).

"The depression arises (as in the mouth region) through increase in the height of the limiting borders. The depression is always closed in by epithelium, and the base of the depression is never separated from the entodermal cloaca by mesodermic tissue." (Born.)

The cloaca is gradually divided into a ventral (bladder-urogenital sinus) segment, and a dorsal (gut) segment. As to the manner of this division, Retterer and Born agree with the Tiedemann-Rathke idea of the gradual separation into two segments through approximation of two lateral folds of mesoderm, while Tourneux believes it to be accomplished by a septal (frontal) downgrowth. Lieberkühn ('82) and Keibel ('89) dispute the theory of Rathke ('32) championed by von Mihlacovics ('85), that the bladder arises from the allantois, and state that it is made up for the most part from the ventral cloacal segment— agreed to by Retterer and Reichel. Born takes a neutral position and believes that at least the trigone of the human bladder may be developed in a manner like that found in the guinea pig (Keibel). Born and Minot do not think that the anlage of the upper part of the bladder is of particular importance. "We are probably not mistaken when we grant that not only the bladder (as far as the apex) but the male urethra as far as the caput gallinaginis, the entire female urethra, and in the male, also the pars prostatica and the entire pars membranacea are developed from the ventral cloacal segment."

The most important of the recent works is that of Ke bel ('96) who sums up the development of the cloacal region in the following words: "The human embryo possesses a large entodermal cloaca in the early stages of its development which however is never opened to the outside through a cloacal anus ('Cloakenafter' of Prenant), but remains closed through the cloacal membrane (the 'anal membrane' of the earlier writers) as long as it exists as such. An ectodermal cloaca is to be found only in traces if at all. The entodermal cloaca is separated into a ventral and a dorsal segment by a frontal septum. A large part or all of the bladder, the urethra and the urogenital sinus as far as the cloacal membrane are derived from the ventral segment; while the dorsal segment becomes continuous with the ectodermal segment of the rectal canal. The primitive perineum is formed when the frontal septum fuses with the cloacal membrane and the rudimentary ectodermal cloaca is then divided by the permanent perineum. The ectodermal anal pit (protodaeum) is situated behind the permanent perineum, while the ectodermal portion of the urogenital sinus is ventral to it."

Another investigator, who has been particularly fortunate in the amount of embryological material at his disposal, expresses a somewhat different view. Nagel ('02) practically reiterates his statements of 1894. "The inspection of the tail end of human embryos of 11–13 mm. length reveals an oval pit extending from the coccygeal prominence to the tip of the genital eminence. This pit (cloaca) receives the openings of the gut dorsally, and the Canalis urogenitalis ventrally; the two separated by a partition of some 0.3 mm. thickness. The Wolffian and Müllerian ducts open higher up in the Canalis urogenitalis and will not be considered in the description of this depression. The Canalis urogenitalis and the gut open into this pit (cloaca) which would reach (comparing with adult relations) from the dorsal border of the anus to the ventral border of the urethral opening (*i.e.* Frenulum clitoridis). Later he states: "In what manner the division of the cloaca is accomplished is not perfectly understood either in man or in mammals. I found the relations in the youngest human embryo that I had opportunity to examine like those pictured in fig. 1, naturally with exception of the form of the bladder. I commit myself therefore, as far as the human embryo is concerned, to the view of Rathke which has recently been substantiated by Retterer and von Mihalcovics in the animals."

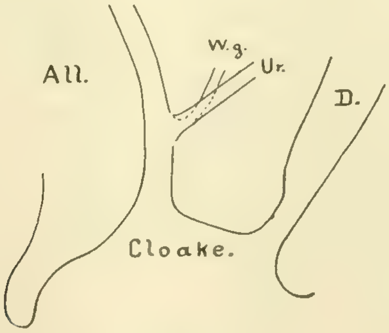

Fig. 1

The work of von Mihalcovics ('85) referred to, says in part; "This septum between the urogenital canal and the gut arises in part through increase in length of the afore-mentioned perineal fold (septum) to which, distalward, two lateral folds join to the perineal folds. The cloaca takes no part in the formation of the urogenital canal. "The opinion of Retterer ('94), mentioned by Nagel, is summarized as follows: "In the guinea pig, as in other mammals studied up to the present time (man, pig, sheep and rabbit), a fold of mesoderm appears at the cephalic extremity of each lateral cloacal wall, and extends little by little toward the caudal end of the cloaca. These lateral folds encroach upon the lumen of the cloaca and divide it into two canals, the one dorsal or rectal, and the other ventral or vesico-urogenital."

That these opinions, while they are in the main contrary to the views of Keibel although they appeared at an earlier date, are accepted at the present time can be illustrated by an extract from Zuckerakndl s Handbuch der Urologie ('03): " In the second fetal month, the proximal segment of the allantois widens to form the bladder, while the distal and narrow portion (urachus) obliterates to form the Lig. vesico-umbilicale." "The division of the cloaca is accomplished by three folds, a median and two lateral. The former occupies the angle between the allantois and hind gut, while the latter are developed in the lateral walls of the cloaca."

The investigations of Tourneux ('94) agree with those of Keibel in that he also states the cloaca to be a closed sac. His objections to the method of cloacal division as described by Retterer are as follows: "The form of the inferior border of the recto-urogenital septum is that of a vaulted arch and not that of an elliptical arch with thevertex upward—afact easily demonstrated in frontal sections. In addition to this, the transverse sections show that the lateral folds are found only toward the summit of the arch and converge rapidly. Further, the septum shows no signs of an epithelial raphe at the supposed line of fusion to indicate the transition that one encounters, as Keibel states, at the line of union of the palatine ridges."

Nagel ('96) answers Keibel's article by stating "If Keibel did not find the facts as presented by myself in all of his embryos of 11.0 mm. and upwards, then the embryos are at fault." "Furthermore in order that an embryo may be declared of scientific value, I demand that the urogenital canal be open into the cloacal pit in all embryos over 8.0 mm., and that the cloacal membrane have disappeared as far as the tip of the genital eminence. Inasmuch as the allantois contained within the umbilical cord is practically obliterated at this stage, where could the secretion from the mesonephros be stored up if the cloacal membrane were intact?"

The questions that appeal to the writer as doubtful ones may be expressed as follows:

1. Does the cloaca proper ever open to the outside?
2. Does the cloacal membrane arise directly by displacement of applied layers of ecto- and entoderm in the primitive streak or is it formed in situ through disappearance of the intervening mesoderm?
3. In what manner is the cloacal division effected?
4. What is the anlage for the bladder?
5. Can the urinary function of the mesonephros assumed by Nagel be demonstrated?

MATERIAL

The study of the cloacal region was done in part by working out the relations in serial sections, and in part through reconstruction. Thirty embryos in all were examined; reconstruction employed in thirteen, of which six stages will be presented. The material, with the exception of these six , is given entirely in the tabulation. The serial number, used throughout this paper, refers to the age of the embryo based on the development of the urogenital tract and with no particular reference to its length. It is interesting to note that with the exception of nos. 4, 11, 13, and 21, the development of the tract has proved to be an excellent check on the determination of the age by the greatest length method. The Mall number refers to the catalogue number of the collection of human embryos at Johns Hopkins University and the section thickness (indicated in microns); the section direction (+ for transverse, = for sagittal, and || for coronal; and the condition (*f* for fair and *g* for good) are recorded in this manner in the Mall catalogue. The modelling of the embryo is indicated by the magnification of the same, and where the model is presented in this paper it is designated by a capital letter. The brief normentafel shows the major points of difference in the development of the cloacal region. The embryo 30 is the Piper II embryo of the collection, and will be presented later in connection with the origin of the bulbovestibular glands. This embryo and also no. 20 have been reported in connection with the condition of complete double ureter (Johns Hopkins Bull., vol. 16, Feb. '04).

TABULATION

Tabulation of Material. + for transverse: = for sagittal: ‖ for coronal. The figures following indicate the thickness in microns.

No. 1 (Mall 12; 2.1 mm.; +; 10; Good.) Modelled by Mall ('97). Allantois of small lumen and in relation with the caudal border of the yolk sac. No evidences of a cloacal membrane.

No. 2 (Mall 391; 2.0 mm.; +; 10; Good). Appearance of hind gut. An interval of several sections between allantois and yolk sac. No cloacal membrane.

No. 3 (Mall 164; 3.5 mm.; +; 20; Good). Model A. × 100. Cloaca well developed and limited for caudal half of the ventral border by epithelial cloacal membrane. Allantois comes off at right angles and at lower border of dermal navel. Lateral furrow along later line of division.

No. 4 (Mall 209; 3.0 mm.; +; 50; Fair). Relations about the same as the foregoing stage. Sections too thick for detailed study.

No. 5 (Mall 186; 3.5 mm.; +; 20; Good). Model B. × 100. Allantois wide lumened and no distinct line of demarkation from the ventral cloacal segment. Wolffian ducts have arrived but not as yet opened. Tail gut maximum of development. Probably the first signs of cloacal division.

No. 6 (Mall 87; 4.0 mm.; +; 20; Fair). About the same as the foregoing.

No. 7 (Mall 148; 4.3 mm.; +; 10; Good × 100). Wolffian ducts open into cloaca. Allantois of small lumen. Ventral cloacal segment widening. Evidences of division quite apparent.

No. 8 (Mall. 76; 4.5 mm.; +; 20; Good). × 100. Widened ventral cloacal segment goes over indistinctly into allantois. Tail gut still quite large. Renal buds well developed. With exception of large allantois and wider tail gut, quite similar to Model C.

No. 9 (Mall 80; 5.0 mm; +; 10; Good). Model C. × 100. About the same as the foregoing stage. Tail gut undergoing degenerative changes.

No. 10 (Mall 371; 6.6 mm.; =; 10; Good). Tail gut rudimentary. Development of cloaca and renal buds about the same as in no. 12.

No. 11 (Mall 116; 5.0 mm.; =; 20; Good). × 100. Intracloacal epithelial plug well marked. Stage about the same as that found in no. 12.

No. 12 (Mall 2; 7.0 mm.; +; 15; Good). Model D. × 100. Marked development of renal buds. Kidney and ureter segments evident. Ventral cloacal segment much widened. Allantois of narrow lumen. Intracloacal epithelial plug marked. Tail gut maximum in length and slightly widened at caudal end.

No. 13 (Mall 241; 6.0 mm.; +; 10; Good). Tail gut more rudimentary than in no. 12. Cloacal membrane wide and shows intracloacal epithelial plug. Otherwise about the same as in no. 12.

No. 14 (Mall 383; 7.0 mm.; +; 10; Good). State similar to no. 12. Cloacal membrane intact.

No. 15 (Mall 397; 8.0 mm.; +; 10; Good). Similar to foregoing.

No. 16 (Mall 113; 8.0 mm.; =; 10= Fair). × 100. Stage of cloacal division between than found in no. 12 and that found in no. 19. Cloacal membrane intact. Division of ureteral pelvis. Ureter segment elongated.

No. 17 (Mall 114; 10.0 mm.; =; 10; Fair). × 100. Stage of cloacal division about the same as the foregoing. Cloacal membrane intact. Kidney a little higher up. Upper and lower pelvis evident.

No. 18 (Mall 109; 11.0 mm.; +; 20; Good). Sections directly through lower part of cloacal region somewhat damaged. Otherwise about the same as no. 19.

No. 19 (Mall 221; 12.0 mm.; =; 20; Good). Model E. × 100. All traces of tail gut lost. Cloacal membrane somewhat depressed from surface. Cloacal segment of Wolffian duct shortened and opening of ureter and duct common into ventral cloacal segment. Beginning formation of genital eminence and lengthening of the ventral cloacal wall. Cloacal membrane intact.

No. 20 (Mall 175; 13.0 mm.; +; 20; Good). × 100. Cloaca about divided. Complete double ureter. Orifices of lateral, and medial ureter and Wolffian duct at same level into cloaca.

No. 21 (Mall 353; 11.0 mm.; +; 10; Good). Cloaca completely divided. Anal and urogenital membranes intact. Ureteral orifice independent and on level with Wolffian opening.

No. 22 (Mall 317; 15.0 mm.; ||; 20; Good). About the same stage as in no. 21.

No. 23 (Mall 350; 15.0 mm; ||; 10; Good). About the same as the foregoing, and no. 24.

No. 24 (Mall 43; 16.0 mm.; =; 50; Good). Model F. × 50. Anal and urogenital membranes separated. Genital eminence more marked. Ectodermic inclusion in furrow on caudal surface. Ureteral orifice on same level but some distance lateral to Wolffian orifice.

No. 25 (Mall 256; 16.0 mm.; =; 50; Good). Urogenital sinus open to outside through rupture of urogenital membrane. Kidney nearly up to normal position and rotated.

No. 26 (Mall 296; 17.0 mm.; ||; 20; Good). Slightly older than above. Appearance of secondary genital folds at the sides of the phallus.

No. 27 (Mall 128; 20.0 mm.; ||; 50; Good). About same as above.

No. 28 (Mall 240; 20.0 mm.; ||; 20; Good).

No. 29 (Mall 22; 20.0 mm.; +; 50; Good). One Müllerian duct has reached the urogenital sinus.

No. 30 (Embryo KP; 22.0 mm.; +; 15; Good). × 66. Both Müllerian ducts at sinus.

The last six embryos of this series have been introduced merely to carry out the idea of a brief arrangement according to the development of the tract in so far as it was possible and to indicate the position of Model of no. 30 referred to in reference to the measurements on the inter-ureteral and inter-Wolffian widths mentioned in the discussion of the anlage for the trigonum. Embryo no. 30 has additional interest in that it like no. 20 has a complete duplication of the ureter on one side.

The description of the models has been made in as brief a manner as feasible, and a discussion of the various points in question will be brought up after they have been described. All of these models represent epithelial casts, and all of them with the exception of Model F. are × 100.

Model A. (*No. 3; Mall no. 164; 3.5 mm.*) This model shows the cloaca to be a sort of conical sac receiving the wider lumened hind gut dorsally, and the narrow lumened allantois ventrally. The latter joining the cloaca at about a right angle. The cloaca is much wider dorsally and is somewhat wedged-shaped in section. Ventrally, it is limited for about one-half of its extent by the epithelial cloacal membrane which is indicated by a surface contact. The right wall of the cloaca is concaved from before backwards while the left side (shown) is correspondingly convex, and presents a well marked furrow extending from the saddle between the allantois and hind gut to about the mid area of the cloacal membrane. This furrow is interesting because while it indicates the probable line of the later division, it was found in but two embryos of the series examined. The part of the cloaca caudal to the cloacal membrane—the tail gut—is quite short and cone-shaped. The model with the exception of the furrow conforms to Keibel's model of the His embryo EB (3 mm.)

Model B. (*No. 8; Mall no. 186; 3.5 mm.*) This model, although made from an embryo of the same length as the above, shows a distinctly older stage in the development of the tract. The allantois has a much wider lumen and the angle between it and the hind gut is much more acute. The Wolffian ducts have reached the cloaca, and attach but do not open into it ventrally near the upper limit of the cloacal membrane. The tail gut is much larger both in length and in thickness, and terminates in an undifferentiated cell mass formed by itself, the chorda and the neural tube (shown as a knob-like ending). The cloaca is wider dorsally than ventrally. A stage similar to the one described was found in two other embryos (nos. 7 and 8), in both of which the allantois was wide at its apparent opening into the cloaca. The possibility of some minor degree of development abnormality suggested itself but on careful study, it was decided that the first evidences of cloacal division were at hand.

Model B, other than a better developed tail gut, a slightly higher position of the Wolffian ducts and a shorter cloacal membrane agrees with Keibel's model of the 4.2 mm. embryo. Keibel's model shows this same evidence of division; a frontal septum between the allantois and hind gut slipping down to a level approaching the upper limit of the cloacal membrane. The writer has found no embryo where the cloacal membrane approaches the level of the dermal navel, and will consider this point later.

Model C. (*No. 9; Mall no. 80; 5.0 mm.*) This model shows marked degeneration of the tail gut. The allantois is narrow lumened, while the widely lumened ventral cloacal segment lies ventral to the hind gut and dorsal segment, and is separated from them by peritoneum. The Wolffian ducts open in the same situation as in the foregoing model, and present dorsal diverticulae, the renal buds. The ventral portion of the cloaca, especially the area above the cloacal membrane, is markedly widened and flattened. The lateral furrow of Model A was not present. This model conforms to the Keibel model of a 6.5 mm. embryo (a considerably older stage if we go by the greatest length rule). The cloaca has lost its more or less even width and attains its greatest lateral diameter at the level of the Wolffian orifices. There is no evidence of displacement of the Wolffian ducts from their primitive position in relation with the upper limit of the cloacal membrane.

Model D (*No. 12; Mall no. 2; 7.0 mm.*) This model fills in a gap in the Keibel series which may be considered quite an important one. The hind gut and all that pertains to the dorsal segment of the cloaca has retained an even caliber, while the ventral segment has widened progressively. The division of the cloaca may be traced to the level of the Wolffian orifices. The Wolffian ducts are much better developed and the renal anlage has resolved itself into distinct ureter and kidney segments. The segment of Wolffian duct between the orifice of the ureter and the cloaca is relatively shorter. The renal anlagen have assumed the position of dorsal convergence (mentioned by Keibel) but this is only a relative matter (see later). The tail gut has undergone further degeneration and has lost its lumen in part.

Model E. (*No. 19; Mall no. 221; 12.0 mm.*) This stage shows the cloaca about half divided along the furrow line indicated in Model A. The cloacal membrane has thickened but mostly through addition of ectodermal cells, and has been displaced through development of the 'precloacal mesoderm' from its primitive position parallel to the dorsal line of the cloaca to one more nearly at right angles to it. In so far as it was possible to ascertain, this downward displacement of the cloacal membrane in no way affects its caudal limit. The dorsal segment has retained the original proportions while the ventral segment has widened — most marked again at the level of the Wolffian orifices. The further development of the kidney and ureter will be noted and the gradual approach of the ureteral orifice to the cloaca proper is evident. The ureter shows signs of shifting from its primitive dorsal position on the Wolffian duct to a more lateral one. The peritoneum has descended to the level of the ducts while the cloacal division is relatively far advanced. The model agrees with Keibel's model of an 11.5 mm. embryo.

Model F. (*No. 24; Mall no. 43; 16.0 mm. × 50.*) Unfortunately this embryo was cut too thick for minute reconstruction. The stage however fils in another gap in the Keibel series. Here the cloaca and the cloacal membrane are completely divided—the ventral segment limited by the urogenital plate and the dorsal segment by the anal plate. The ureter is displaced from its dorsolateral position on the Wolffian duct to a supero-lateral one and opens distinctly into the ventral cloacal segment. The marked increase in precloacal tissue has resulted not only in the large genital eminence, but the urogenital plate has been dislocated deeper into the base of the phallus and a marked heaping up of ectodermal cells has occurred in the furrow on the caudal surface of the eminence. The two resultant segments of the cloacal membrane, the urogenital and anal plates are apparently no longer than they were in much younger stages; a point that will be brought out in greater detail later.

THE CLOACAL MEMBRANE

While authorities seem to agree that the cloacal membrane arises from the primitive streak, or at least that portion which has been displaced ventrally in the formation of the tailfold, they disagree on the point whether this part of the primitive streak consists in applied layers of ecto- and entoderm, or whether it is composed of all three germ layers. Stated in an simple manner, is the cloacal membrane formed dorsally as an epithelial membrane or is it formed ventrally through disappearance of intervening mesodermal tissue? One of the earliest stages in the formation of the tail fold is found in the Spee reconstruction ('96) of embryo Gle (2.0 mm.). A schematic sagittal section is presented in his fig. 1, and emphasis is laid on the point that the primitive streak is composed of all three germ layers. No. 1 of the present tabulation was reconstructed by Mall ('97), and while a slightly older stage in the development, shows the cloacal sac limited ventrally by all three germ layers. No. 2 presents a similar condition. The first embryo of this series to show a cloacal membrane is no. 3 (3.5 mm.) which is quite a little farther advanced in the development than no. 2; unfortunately the transition stages are wanting. It is the writer's opinion therefore that the view held by Keibel is the correct one, and that while the cloacal membrane is derived from the primitive streak area, it is formed in situ as an epithelial membrane on the ventral cloacal surface, naturally after the formation of the tail fold.

The sagittal width of the cloacal membrane varies somewhat with the stage in the development. Born's statement that it extends as far cephalward as the dermal navel is based on Keibel's model of a 4.2 mm. embryo ('88). A somewhat similar condition is noted in two of the embryos of this series. The writer does not believe this to be the normal relation, but rather one which through persistence may have a decided bearing on bladder exstrophy and epispadias. The epithelial plate, if it extends to the dermal navel, and persists in this relation, separates the precloacal tissue into two lateral halves, and accounts quite satisfactorily for the deficience in the abdominal wall and for the gutter on the upper surface of the phallus. In practically all of the embryos examined, the cloacal membrane did not extend higher than the level of the Wolffian orifices. The membrane is normally shortest in length at the time of its formation but attains its greatest length in Model C. (0.29 mm.); in Model D (0.27 mm.); in Model E (0.34 mm.). This increase is not marked when we consider that these stages represent 5, 7 and 12 mm. embryos respectively.

The width of the cloacal membrane is a rather indefinite quantity to measure; it widens progressively however with the widening of the ventral cloacal segment, and in the proportion of 5 in B to 8 in C to 12 in D or in other words in proportion to the greatest length measurement of the embryo.

The position of the cloacal membrane is about parallel to the long axis of the embryo in Models A, B and C. With the development of the precloacal tissue, the cloacal membrane is displaced so that its upper end is pushed caudalward—beginning in D; more marked in E; and culminating in F where the upper end of the membrane has been displaced through practically 90° with the caudal end as the fixed point of the radius. The displacement is probably due to the active lengthening of the ventral cloacal wall and to the development of the genital eminence. The membrane is divided into two parts in Model F—a ventral or urogenital membrane and a dorsal or anal membrane; both of which have been displaced from the surface in the manner suggested by Born. The anal membrane persists beyond the stages described in this paper, and is dealt with in sufficient detail in Keibels' article. The urogenital membrane ruptures with its formation and is of particular interest in its relation to the position of the bulbovestibular glands; a point to be brought in a paper which is to appear shortly. It may be noted that in Model D the cloacal membrane is much thicker in its midarea, and not infrequently one finds a heaping up of cells in this region and a corresponding eminence on the inner surface of the cloacal (intracloacal epithelial plug). The significance of this is not understood but it may be due to the more active proliferation of the ectodermal cells which contribute to the formation of the cloacal membrane.

THE ECTODERMAL CLOACA

An ectodermal cloaca has been stated by Keibel to be present only in traces, if at all. None of the embryos examined showed a surface depression, and we may therefore critically examine Nagel's fig. 1 and his contention concerning the cloacal membrane. It will be seen that in fig. 1, while the embryo shows a cloacal division comparable to our Model E; the phallus is developed to the extent of that found in Model F; while the cloacal membrane is ruptured. None of the embryos of this stage, or younger than this stage, showed rupture of the cloacal membrane, and we may therefore agree with Keibel that the cloacal membrane remains intact normally as long as it persists as such. Nagel believes that the rupture of the membrane is essential or where would the secretion of the mesonephros be passed? We may answer this question. The ventral cloacal segment and the allantois do not show the degree of widening necessary to accommodate the urinary excretion, and we call to mind Hill's work on the pig embryo ('04) and our own ('04) on the human embryo, in both of which it is conclusively shown that the mesonephros is actively degenerate long before the kidney is developmentally fitted to take up a urinary function. Cases of atresia urethrae commonly occur without bladder distension; monstrosities may go to term without kidneys and with no undue persistence of the mesonephros; and experimentally, it has been shown that there is no evidence of urinary excretion into the amniotic fluid even in term children. We do not believe it necessary to assume that the mesonephros functionates as an organ of excretion in the placental mammals, and do not consider Nagel's contention for a functional rupture of the cloacal membrane as a serious one.

THE TAIL GUT

The tail gut is that portion of the enteron that lies caudal to the lower limit of the cloacal membrane and before the formation of the cloacal membrane has no definite line of demarkation from the cloaca proper. The tail gut is represented by a conical sac in

Model A and attains its largest proportions in Model B or at about the time that the Wolffian ducts reach the cloaca. Its relation to the neural tube and to the chorda is most marked at this stage in the development but the histogenesis of this region (shown in Model B as a terminal knob) together with its possible connection with the ano-coccygeal body is not definitely known. The degenerative changes have already set in in Model C and are more apparent in Model D. The shrinkage in size does not affect the tail gut evenly throughout its length, but is less marked at the caudal extremity where a cord of cells may be found even after all traces of the primitive connection with the cloaca have disappeared. In general, the tail gut arises with the tail and accompanies the tail in its development and resorbtion. It appears at a 3.5 mm. stage and has almost completely disappeared in an 8.0 mm. embryo. The comparative embryology of this rudimentary and transient segment of the gut deserves careful study.

THE CLOACA

Morphologically, we may hardly consider that portion of the gut caudal to the orifices of the allantois and hind gut as cloaca until the arrival of the Wolffian ducts contribute a connection with the urogenital system, and practically we may not consider the sac as such until the formation of the cloacal membrane indicates how much is cloaca and how much is tail gut. The condition in the development which antedates these structures might be termed the precloacal stage.

Born states that the allantois arises ventrally in the human embryo and in close relation to the yolk sac, and that the allantois is displaced caudalward through growth in the intervening mesoderm. We may agree with the first part of this statement from the relations demonstrated in the Spee embryo and in Mall's reconstruction of no. 1. The last point, the downward displacement of the allantois, may be seriously questioned. In the formation of the hind gut, either the allantois is displaced caudalward, or the yolk sac is pushed upward on the gut. A comparison of embryos 1 and 3 favors the latter view. The development of the hind gut does not seem to take place at the expense of the cloaca, but the allantois appears to occupy a relatively fixed position at the lower border of the dermal navel. This argument is strengthened by the active increase in length of the hind gut, and by such conditions of strictly pathological nature such as bladder exstrophy. The line of demarkation however between what is allantois and what is ventral cloacal segment is largely a matter of topography, but is after all of some importance in a definite determination of the anlage for the urinary bladder.

THE DIVISION OF THE CLOACA

The division of the cloaca manifests itself in a separation into ventral and a dorsal segment. According to Tourneux, the division is accomplished through the downgrowth of a single mesodermic fold (the saddle between the allantois and hindgut), while Retterer maintains that two lateral mesodermic folds encroach upon the lumen of the cloaca and pinch it off, as it were, into the two resulting segments. Minot ('97) and Keibel hold the manner of division to be of little moment, and even Fleischmann ('07) appears to have given up hope of solving the question from the standpoint of comparative embryology. Tourneux's idea is, according to the writer, a much better description of the process than that of Retterer.

Taking the Wolffian ducts as a fixed point, it is easily seen that a coronal septum grows down dorsal to the Wolffian orifices until finally the entoderm covering the septum comes into relation with the entoderm of the cloacal membrane. With the disappearance of the epithelium in this fused area, the mesoderm of the setpum touches the ectoderm of the surface and the cloacal membrane is split into its two resulting segments. While this division of the cloaca results in two segments that are about of equal size as far as their antero-posterior measurements are concerned, and inasmuch as the cloacal membrane does not materially increase in length, it may be assumed that the cloacal membrane is split into about equal parts in its transformation into the urogenital and anal membranes. This would mean that the increase in the apparent length of the urogenital membrane is due to proliferation of the ectodermal cells or to inclusion of the surface ectoderm as Born states it. The difference in opinion on this point will be brought out in a later article.

The development of the ventral cloacal segment interests us because it has to do with the formation of the bladder. Detailed measurements of this region have been suppressed because of the variable size of the cloaca proper, but the union of the allantois to the cloaca seems to be fairly definite in Models C, D and E. In all of these embryos the Wolffian ducts open a little above the center of a line drawn from the caudal extremity of the cloacal membrane to the apparent line of union of allantois with cloaca. We have therefore no material reason for not believing, inasmuch as this is the primitive relation of the Wolffian orifices and there is no evidence of downward displacement of the allantois, that the gradually constricting area above the Wolffian orifices is ventral cloacal segment (see Model B).

The active widening of the ventral cloacal segment, particularly at the level of the Wolffian orifices, is evident to every observer of the cross sections in this region, and in the dorsal convergence of the ureters—a fact first noted by Lieberkühn and Keibel. It is evident also, as suggested by them, that the distal segment of the Wolffian ducts may become incorporated into the anlage for the bladder. This alone does not however account for the lateral rotation of the ureter to gain the position noted in Model F, nor does it explain the later upper displacement of the ureter to its normal position in the bladder.

It is seen in our models that the renal buds appear on the dorsal aspect of the Wolffian ducts at some distance from the cloaca,and the intervening segment of duct may be termed the 'cloacal segment' merely for convenience in the description. It is well known that this gradual shifting of the ureter is not due alone to the resorption of the cloacal segment of the Wolffian duct, for when two ureters arise from the same duct, the one with the orifice nearest the cloaca assumes the more lateral position. In this region we have to deal with the relations as they appear from their topography for it is not possible as yet to differentiate mesodermal epithelium from that of entodermal origin.

The widening of the ventral cloacal segment is made evident from the following measurements. A comparison of the inter-Wolffian width and the width of the dorsal segment gives a proportion of 20 : 9 in B; 28 : 8 in C ; 36 : 9 in D ; and 46 : 9 in E. This means that the ventral cloacal segment increases markedly in width while the dorsal segment remains about the same. Observed in transverse sections, this active widening gives the impression of two lateral mesodermic folds encroaching upon the cloacal lumen (Retterer's idea.) The widening of the ventral cloacal segment is most marked at the level of the Wolffian orifices and progresses equally both upward and downward giving the idea coronal section of the cloaca in Model E a diamond shape.

Born has stated that the trigonum may be of mesodermic origin. We have measured Model F and compared it with Model 30 and find the following: that the inter-ureteral distance (between the orifices) is greater in F (0.55 mm.) than in no. 30 (0.34); while the Wolffian ducts in the former are almost twice as far apart as in the latter. This implies that the ureter is not displaced lateralward from the duct but that both are displaced medianward, and that if there is any part of the bladder to which the mesoderm has definitely not contributed it is the trigonum.

We are of the opinion that the ventral segment of the cloaca probably forms the anlage for the bladder including the urachus, as was stated in Born's article, and that the cloacal segment of the Wolffian ducts (mesodermic) contributes some part. How much is impossible to determine. When the cloacal membrane extends as far upward as the dermal navel and persists as an epithelial membrane, we have the origin of the cases of complete bladder exstrophy which take in the whole ventral wall of the bladder and urachus. The present champion of the allantoigen origin of the bladder is not supported by our investigations. Rather we agree with Disse ('02) who states that Nagel misunderstood the relations found in the earlier stages through examination of embryos too far advanced in the development.

In conclusion we may sum up the points that have been investigated in this paper in a review of the development of the cloaca and of the structures in relation with it. The article has been written largely as a check on the earlier stages in the development in order that the writer might have first hand information regarding this important segment of the enteron.

SUMMARY

The cloaca is a closed entodermal sac and is never normally opened to the outside (amniotic cavity) through rupture of the cloacal membrane. It represents that portion of the enteron which lies caudal to the opening of the allantois and may not be separated from the tail gut until the formation of the cloacal membrane, when the inferior border of the membrane forms an arbitrary limit between the cloaca and the tail gut. The cloacal membrane arises from the primitive streak area. The primitive streak at the time of its ventral displacement in the formation of the tail fold, is composed of three germ layers, and the cloacal membrane is formed in situ through disappearance of the intervening mesoderm. Normally, the cloacal membrane occupies the caudal half of the ventral cloacal border, but in some cases, the membrane may extend as far cephalward as the dermal navel. This latter condition has an important bearing on the embryology of bladder exstrophy and epispadias. The increase in width and thickness of the cloacal membrane is concomitant with the corresponding changes in the ventral segment of the cloaca, and is due to ectodermic proliferation. The membrane is displaced in the process of cloacal division through development of the precloacal mesodermic tissue to form the genital eminence, and at the same time, the original membrane comes to lie deeper in the substance of the embryo. The division of the cloaca begins before the arrival of the Wolffian ducts, and is effected by the downgrowth of a coronal septum. The division, when completed, results in a ventral, (or bladder-urogenital sinus,) segment and a dorsal, (or rectal,) segment which are about of equal size as far as their antero-posterior measurements are concerned. The same holds true of the resultants of the division of the cloacal membrane—the urogenital and anal membranes; both of which are about of the same length and both of which are displaced from the surface through upgrowth of the surrounding mesoderm. The ventral cloacal segment enlarges progressively in length and in width during the division of the cloaca, and as the widening is most marked at the orifices of the Wolffian ducts, the cloacal segment of the ducts may contribute to this active enlargement, or in other words, some of the epithelium of the bladder may be of mesodermic origin. The displacement of the ureter on the Wolffian duct, and the later upward displacement of its orifice from that of the duct, is due in part to the disappearance of the cloacal segment of the duct. The orifices of both ureter and Wolffian duct are however shifted medianward after the completion of the cloacal division, so that the trigonum may be spoken of as distinctly of entodermic origin. The greater part of the bladder and urachus are developed from the ventral segment including the mesodermic contributions from the Wolffian ducts. The allantois probably contributes no part in the formation of the bladder and retains its original relation to the lower border of the dermal navel. The urogenital membrane ruptures normally when the division of the cloaca is completed, or in other words, with its formation, and before the arrival of the Müllerian ducts at the urogenital sinus. The contribution of the entoderm and ectoderm in the formation of the urethra, together with the developmental relations of the bulbovestibular glands, will be considered in a later paper.

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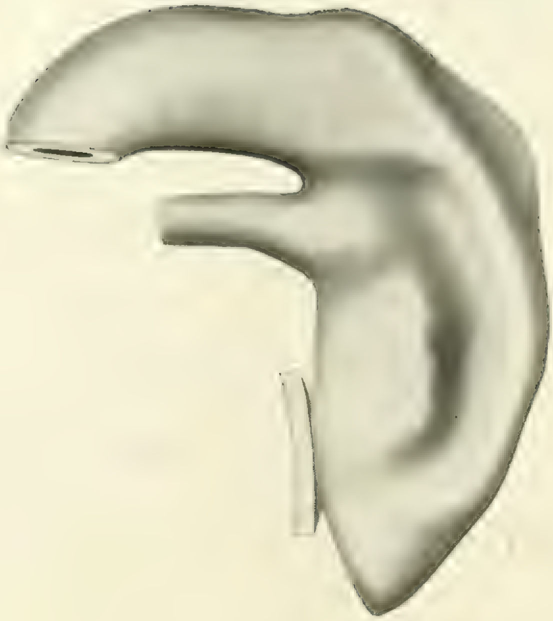
KEY TO LETTERING

A., Allantois
A.M., Position of anal membrane
C., Cloaca
C.M., Cloacal membrane
C.S., Cloacal segment of Wolffian duct
G.E., Genital eminence
H.G., Hind gut
K., Kidney
P.T., Precloacal mesodermic tissue
R.B., Renal bud
T.G., Tail cut
U., Ureter
U.G., Urogenital sinus
U.M., Position of urogenital membrane

* Probable position of point where allantois joins cloaca.

All drawings represent 100 diameters enlargement except of Model F which is 50 diameters.



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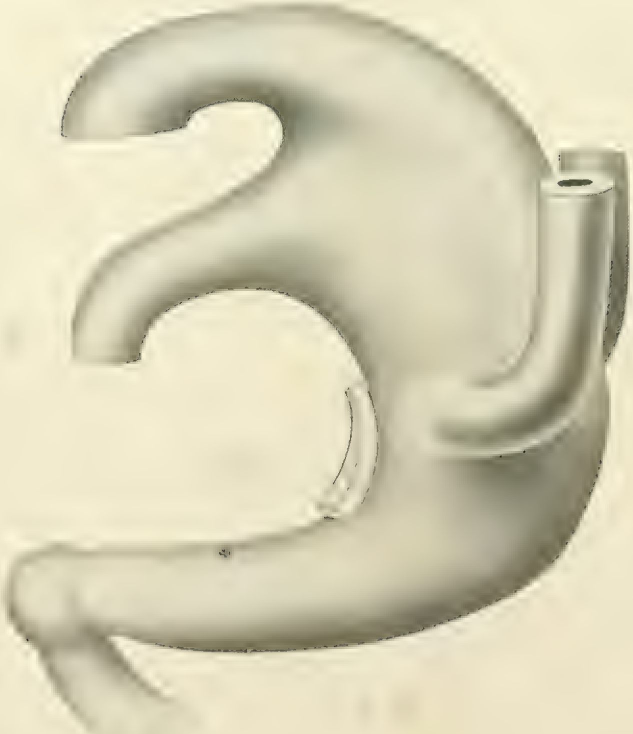

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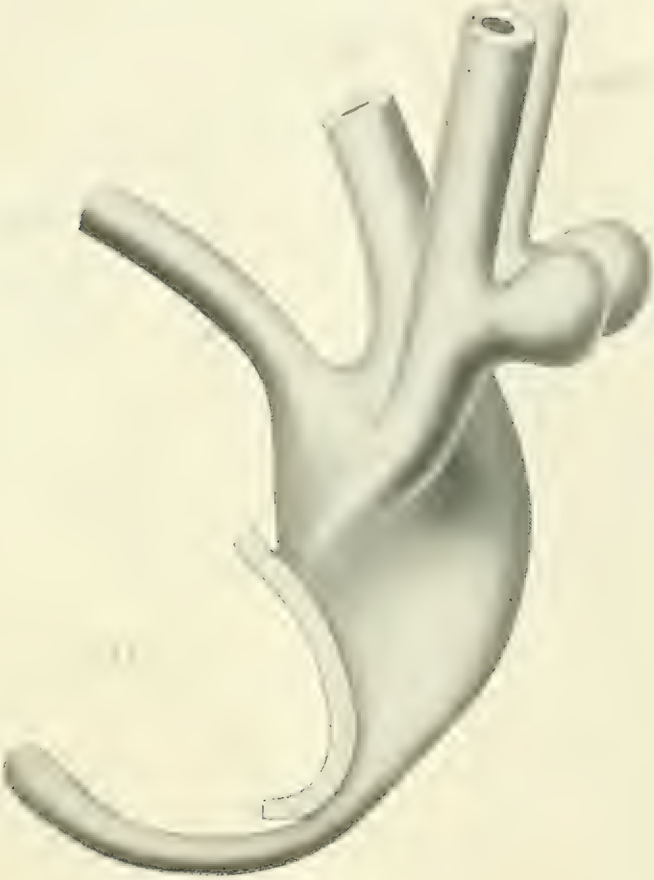

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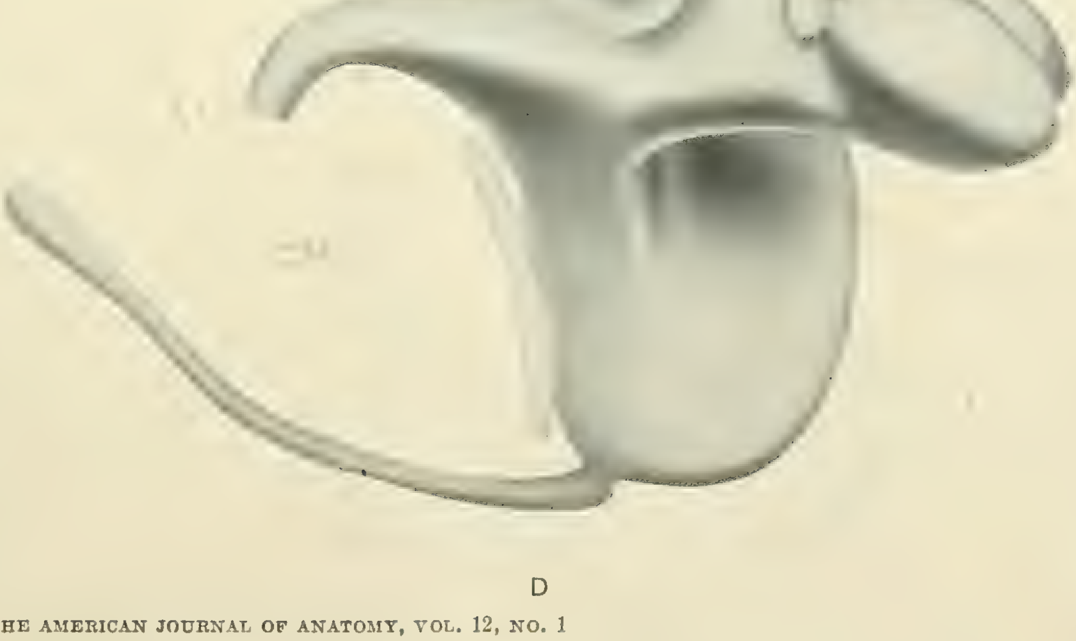

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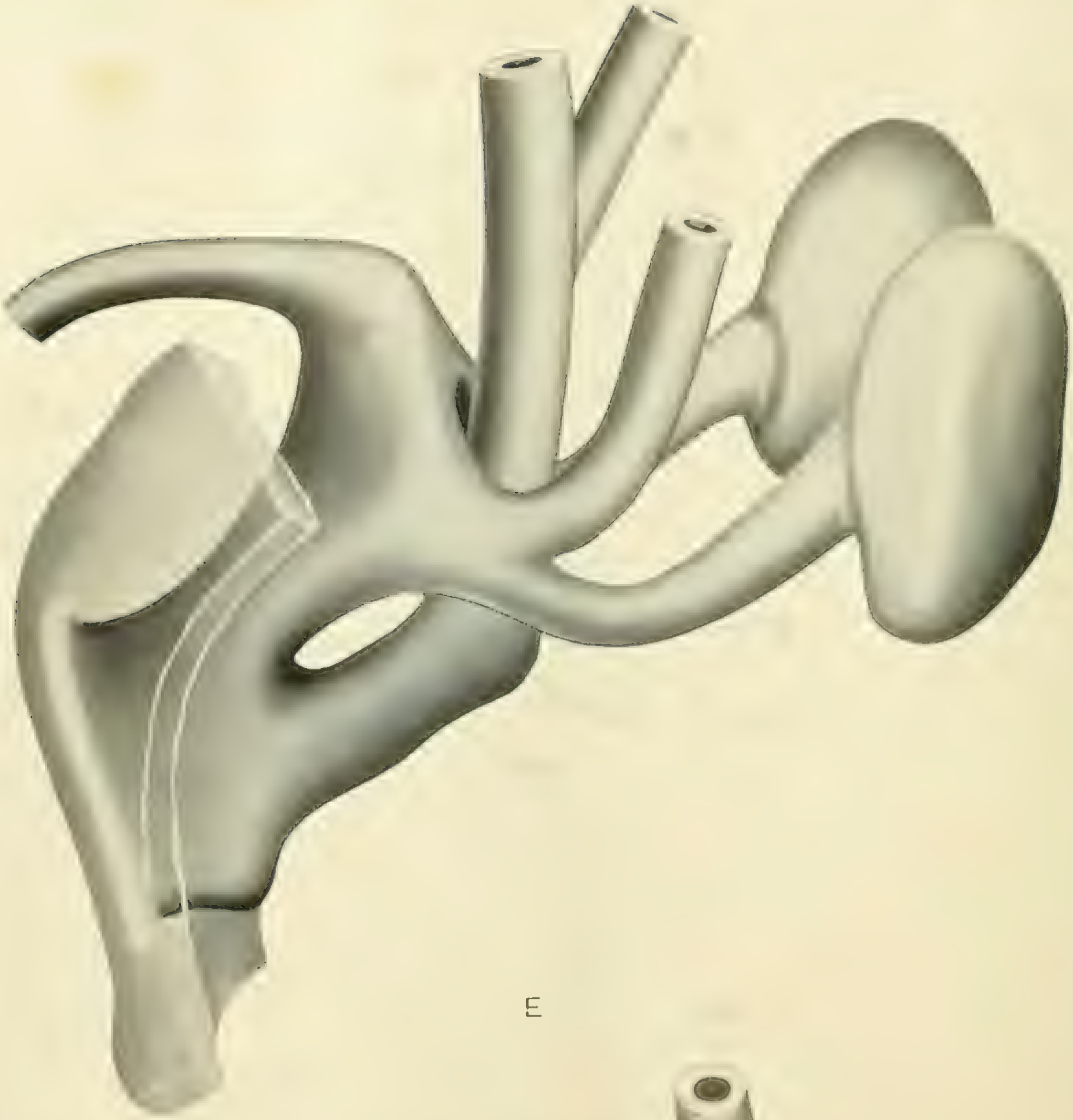

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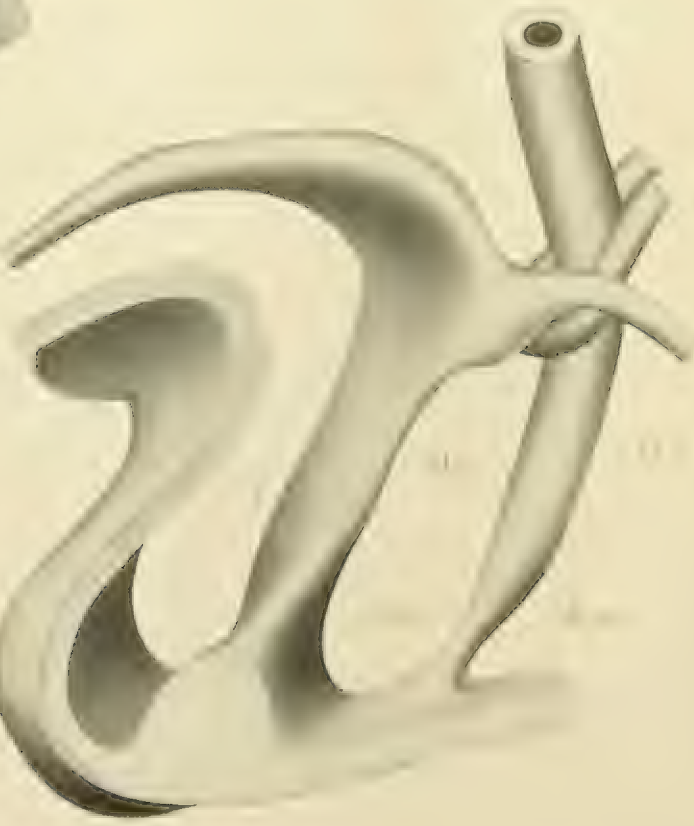

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STUDIES ON THE DEVELOPMENT OF THE HUMAN LARYNX

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THIRTY-NINE FIGURES

ON THE DEVELOPMENT OF THE HUMAN LARYNX

The investigation of the embryonic larynx has been by no means neglected. It has received the attention of many investigators resulting in several valuable contributions. Either by reason of limited material or of especial interest in particular features, these researches have usually been directed into the consideration of only parts of this complex field. There is still lacking a well rounded comprehensive review of the whole subject. Nor does this paper propose to accomplish this. The scope has been limited to a study of the cartilages, muscles, and nerves, during that period of embryonic life, when the most active development occurs. This comprises those stages where these respective elements are first definitely recognizable, to where they assume more or less, their adult relationship, namely, from the 10.5 mm. (5 weeks?) human embryo to the 20 mm. (7½ weeks?) human embryo. Investigations on earlier stages although perhaps somewhat problematic in results have been made, and excellent accounts of still later development can be found in the literature.

METHODS AND MATERIALS

The material at my disposal was Dr. Mall's collection of human embryos, for the use of which I am sincerely grateful. Likewise at this time, I desire to express my appreciation of the interest and advice of Dr. Warren H. Lewis, under whose direction these studies were undertaken, and to Mr. Max Broedel for valuable suggestions regarding some of the illustrations.

I have included a large number of drawings in the hope that they would help to make lucid the descriptions of the various structures involved. Accuracy and fidelity to the original sections has always been the main object. However, it must be said, that in reproducing embryonic tissue, peculiar difficulties are encountered. Condensed mesenchyma is not as a rule clearly outlined, but shades gradually into the surrounding tissue, so that some exaggeration is permissible, and in fact necessary in giving sharp clear outlines to such structures for proper interpretations and the construction of models.

The embryos were cut in various planes, sagittal, transverse, and frontal, thereby affording better opportunities for study. They include the following:

EMBRYO	LENGTH	THICKNESS	SECTIONS
	mm.	μ	
109	10.5	20	Transverse
317	12.5	50	Frontal
144	14	50	Sagittal
43	16	50	Sagittal
128	19.5	50	Frontal
22	20	50	Transverse

Each section in the laryngeal region was carefully studied, projected to sufficient magnification, and the different structures outlined and identified. Recourse was then had to wax model reconstruction, and to the several well known graphic methods of reconstruction.[1]

I have purposely omitted any consideration of the vascular development of this region, as reconstruction methods in embryos are somewhat hazardous for such purposes. The modern injection methods are far more accurate. Much remains to be done in this section.

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[1] 24th Session. Amer. Assoc. of Anat., Baltimore, Md., December 1908–January 1909. Lisser: Models to show development of human larynx.

In considering the musculature of the larynx, it soon became evident, after dissections and studies of the adult larynx, that there was by no means unanimity of opinion among different authorities as to just what constituted the muscles of the larynx. Of course, every one recognized and mentioned the cricothyreoideus, the cricoarytaenoideus posterior and the cricoarytaenoideus lateralis. But some spoke of an inter artyaenoideus muscle, others divided this into an arytaenoideus transversus and artyaenoideus obliquus. Some added an aryepiglotticus and a thyreoepiglotticus; others merely pictured one of these, and some, neither of them. Others again, divide the thyreo-arytaenoid into an internus and externus, and Sewell Seymour ('05) has dissected a 'small or superficial thyreo-arytaenoid muscle' which, moreover, he differentiates into four types. It is therefore apparent that there is not yet a clear cut conception of the exact musculature of the normal adult larynx. Certainly, if such difficulties present themselves in the adult fully formed larynx, how much more confusion would there be in attempting to isolate these varieties in a human embryo. So that I arbitrarily selected the following nomenclature for the laryngeal musculature, as being the simplest and most reliable:

M. m. cricothyreoideus, cricoarytaenoideus posterior, cricoarytaenoideus lateralis, thyreoartyaenoideus, interarytaenoideus, aryepiglotticus, and thyreoepiglotticus. I have purposely regarded the thyreoarytaenoideus as a single muscle, because any demarcation between an externus and internus and a small or superficial, though interesting, is quite artificial, not conclusively demonstrated, and not warranted by the existence of separate distinct action of such subdivisions. The advantage of this classification has been borne out by the following embryological studies, where each of the above enumerated muscles has been clearly found, and none of the other varieties has been represented, though carefully searched for.

Embryo 109–10.5 mm. Transverse sections, 20 μ

(Embryo 109 measures V. B. 10.5 mm. and N. B. 11 mm. in length and is about 5 weeks old.)

THE CARTILAGES

The cartilages of the larynx, or better what become the cartilages of the larynx, are at this stage but very imperfectly formed, as is to be expected, since they reveal themselves for the first time in a 10.5 mm. human embryo. Consequently their appearance is introduced by condensations of mesenchyma, very well termed "pre cartilage." No true cartilage formation, whatsoever, has occurred at this time in any of the laryngeal cartilages, nor yet in the hyoid bone and styloid process. I have investigated the sections of 8, 8.5, 9, and 10 mm. embryos for earlier traces of these structures, but have been consistently unsuccessful, so that I place the first recognizable stage of the larynx skeleton (if one may be permitted to use such an expression) at 10.5 mm. in man. Of course, it is well known that all the structures of the larynx have their ultimate foundation in the gill arches, and I refer those interested in this very early stage of the subject to Frazaer's interesting studies; but I would be skeptical of finding, with the present means of study at our disposal, actual structural status, as regards separate cartilage and muscle masses, earlier than 10.5 mm. in man.

The cricoid-cartilage. (*Figs. 1–2.*) Contrary to expectations, the cricoid cartilage does not appear to develop from two lateral portions of condensed mesenchyma, separate and independent of each other, which later grow together ventrally and dorsally about the lumen of the larynx; nor as one might be led to anticipate, form a large area posterior or dorsal to the larnyx lumen (this portion later becoming more prominent by far than the anterior arcus). But at this stage, there is a predominance of condensed mesenchyma about the ventral portion or arcus, which fades off laterally, and then becomes more emphasized again, by greater compactness and deeper stain, dorsal or posterior to the lumen, but not so extensively nor so well marked as ventrally. This is true provided one considers the deeper staining, more strikingly isolated portions of condensed mesenchyma, as the anlage. So that it seems reasonable to assume that the cricoid cartilage originates from an anlage primarily ventral in the position of what later becomes the anterior arcus, and also, though less prominently from a posterior portion, of itself perhaps, originating from two slightly separated posteriorly lateral portions. The lateral portions then, develop by a welding of the anterior and posterior portions lateralward. At this stage then, the ventral portion of the cricoid cartilage is appreciably advanced in development over the rest of this structure. It does seem difficult to reconcile this with the appearance, for instance, in the 20 mm. stage, when the ventral portion still persists as condensed mesenchyma, while the lateral parts have undergone considerable chondrification.

The arytaenoid cartilages. It is doubtful whether these structures can be determined at this early period in development; probably not. There are two faint, indefinite masses which suggest beginning condensation, but as there is a possibility of these being a part of the superior portion of the cricoid, no positive assertions can be made as to their independence.

Thyreoid cartilage. This cartilage like the cricoid, makes its initial appearance at this stage. I have looked in vain for its rudiments in earlier human embryos. There has been quite a lengthy discussion, during most of the nineteenth century, in which many have participated, as to whether the thryeoid cartilage develops from two lateral anlages which grow around and fuse ventrally, or whether there is in addition to these lateral anlages, a third one a pars intermedia. Nicolas, in his excellent resumé of the subject treats this very fully. My observations inclined to the former view, as in all the stages studied, the lateral portions depict a decidedly more advanced stage of development; yet, I must add that I have found no stage, where the lateral halves alone were present, and in which there was no indication whatsoever of a ventral condensation. Fig. 2 shows the thyreoid cartilage as it exists in a 10.5 mm. embryo. It will be noticed that there is no interruption in the continuity between the two lateral portions via the ventral part; but it will also be seen that the lateral parts exhibit a denser condensation. There is no suggestion of an inferior or superior cornu. The thryeoid cartilage merely looks like a horse shoe mass of condensed mesenchyma.

The hyoid bone and styloid process. These bodies are easily made out existing as pure condensed mesenchyma. .The styloid process is more advanced than the greater cornu of the hyoid at this time.

The epiglottis. This cartilage can be discerned, much flattened in comparison with the adult type. It shows as a beginning area of condensation.

THE MUSCULATURE

The eight muscles are first visible at this period of development, not all as individual muscle masses however, although it is true that one or two of the muscles can be clearly differentiated from the rest. It is curious to note that the laryngeal musculature shows more advanced differentiation than the pharyngeal constrictors, as shown by clearer outline and more extensive fibrillation.[2] The intrinsic œsophageal musculature however is farther developed. The sphincter formation, to which attention has been called by so many writers, can be recognized at this stage, and in a few sections there is some tendency to continuity between the fibres of the outer pharyngeal ring and the inner laryngeal one. Probably too much stress has been laid on this structure, perhaps by reason of the fact that an analogous muscle has been found in lower animals. At any rate, the muscles of the larynx differentiate themselves much earlier than has been previously believed. For instance, the crico artyaenoideus posterior (fig. 1) is unmistakably isolated at this stage, well defined of good size, and abundant fibrillation; nor does it include any other laryngeal muscle. There is also a muscle mass with some fibrillation, not so large, but plainly evident, which apparently includes the crico arytenoideus lateralis and thyreo arytaenoideus (fig. 2), principally the former. It is placed on the lateral surface of the cricoid cartilage. The interarytaenoideus; if it exists at all, does not appear as one muscle. In the position where one would expect to find it, there is continuity of the larynx and pharynx lumina. But just at the point where these join there are muscle fibres on either side, but which do not unite. These may later bridge across to form the m. interarytaenoideus, but of that point, I am by no means certain. No trace was found of the m. aryepiglotticus or the m. thyreoepiglotticus. The m. cricothyroideus is fairly well developed, though not nearly so far advanced in form and size as the m. cricoarytaenoideus posterior. It is the only one that shows any tendency to relation with the pharyngeal musculature, and Frazaer considers them to have the same origin. In general the musculature of the larynx is rather better defined than the cartilages at this period. Strazza in 1888, completed the only really valuable work done on the development of the human laryngeal musculature. Nothing of importance has been added since his paper. He thinks that the laryngeal, tongue and pharyngeal musculature develop out of one and the same muscle mass, which in the early embryo develops from an isolated 'muscle island,' which exists independently of the muscle plates. And that the premuscle tissue of the tongue and larynx is a continuous one, the latter merely lying inside the former. In the region of the epiglottis and larynx, he thinks, is also contained the premuscles masses, though he cannot differentiate them at all in his youngest embryo (12 to 13 mm.). He associates the simultaneous development of the tongue and larynx musculature from the same source, with the fact of the union in speech between the muscles of the tongue and larynx. This is an attractive theory, but my observations cannot substantiate his statement. There is no indication that the larynx muscles develop from the myotomes, on the contrary, they apparently arise from the ventral visceral mesenchyme which continues up into the floor of the mouth. *Bvt in this 10.5 mm. embryo in which even certain larynx muscles can be isolated, there is no association with the tongue musculature, and but little with the pharyngeal set.* In earlier stages the cells which are to form the premuscle masses cannot be distinguished by our present methods from other cells of the condensed mesenchyme of this region.

THE NERVES

The n. laryngeus superior can be traced to the vicinity of the greater cornu of the hyoid, and the wing of the thyreoid, but I could not follow either the motor branch to its inervation of the crico thyreoideus muscle, or the sensory portion, within the larynx. Bits of tissue, were seen that might be nerve tissue, but I cannot be certain of this branch of the vagus any further than to its proximity to the hyoid and thyreoid. The nerve Recurrens, later the nerve laryngeus inferior (fig. 1) is better developed and can be followed clearly to its innervation of the crico artyaenoideus posterior; but I cannot trace it to the other muscles, nor to any anastomosis with the n. laryngeus superior.

Embryo 317—12.5 mm. Frontal sections—50μ

THE CARTILAGES

The thyreoid cartilage still consists purely of precartilage,—condensed mesenchyma, and has not changed greatly in appearance from the one in the 10.5 mm. human embryo. However, rudiments of a superior cornu and inferior cornu are just discernable, and the lateral halves are not quite so rounded as a part of the horseshoe arrangement, but rather more vertical as in fig. 3. The ventral condensation is again in perfect continuity with the lateral parts. This ventral portion is at a lower level than in the adult, coming in close contact with the body of the cricoid, especially its anterior arcus. The lateral wings are well removed from the lateral portions of the cricoid, and between them is ample room for the lateral larynx muscles; the circothyreoideus, circoarytaenoideus lateralis, and perhaps thyreoarytaenoideus.

The cricoid cartilage consists likewise of condensed mesenchyma but is somewhat ahead of the thyreoid in assuming definite shape; certainly the intensity of the condensation is greater, as determined by deeper staining and clearer outline; the ventral arcus maintains its lead over the dorsal and lateral portions although the latter show definite increase in the size of the condensed masses

LIST OF ABBREVIATIONS

V. C., vertebral column
Esoph. L., lumen of esophagus
L. L., larynx lumen
B. C., buccal cavity
Ph. L., pharynx lumen
S. N. S., sympathetic nervous system
Tr., trachea
constr., constrictor muscle
m. constr., middle constrictor muscle
inf. constr., inferior constrictor muscle
cri. post., M. cricoarytaenoideus posterior
cri. lat., M. cricoarytaenoideus lateralis
thy. ary., M. thyreoarytaenoideus
cri. thy., M. cricothyreoideus
int. ary., M. interarytaenoideus
ary. epi., M. aryepiglotticus
thy. epi., M. thyreoepiglotticus
thy. hy., M. thyreohyoideus
omo. hy., M. omohyoideus
mylo. hy., M. mylohyoideus
sternothy., M. sternothyreoideus
sternohy., M. sternohyoideus
dig., M. digastricus
gen. gl., M. genioglossus
gen. hy., M. geniohyoideus
esoph. m., esophageal musculature
ary. m., arytaenoid masses
cri.c., cricoid cartilage
thy. c., thyreoid cartilage
hy. b., hyoid bone
g. c., greater cornu, hyoid bone
sty. p., styloid process
epig., epiglottis condensation
ary. c., arytaenoid cartilage
sup. c., superior cornu, thyreoid cartilage
inf. c., inferior cornu, thyreoid cartilage
Meck. c., Meckel's cartilage
occ. b., occipital bone
tr. c., true cartilage
pre. c., pre-cartilage
c. s. thy. c., cut surface thyreoid cartilage
n. v., nerve recurrens
n. XII., N. hypoglossus
sup. lary., N. laryngeus superior
n. x., n. vagus
ana., anastomosis between superior laryngeal and inferior laryngeal nerves
m. br., motor branch of superior laryngeal nerve
thy. gl., thyreoid gland
s. m. gl., submaxillary gland
s. m. gangl., submaxillary ganglion
p. thy., parathyreoid gland
thy. hy. l, thyreohyoid ligament
car. a., carotid artery.
ant. card., anterior cardinal vein

Fig. 1 Cross section of human Embryo no. 109 (10.5.) to show cricoid cartilage and M. cricoarytaenoideus posterior. *V. C.*, vertebral column; *Esoph. L.*, Lumen of esophagus; *constr.*, constrictor muscle; *cri. post.*, M. cricoarytaenoideus posterior; *n. r.*, nerve recurrens; *cri. c.*, cricoid cartilage; *thy. gl.*, thyreoid gland; *B. C.*, buccal cavity; *L. L.*, larynx lumen.

Fig. 2 Cross section of human Embryo no. 109 (10.5mm.) to show thyreoid cartilage, M. cricoarytaenoideus lateralis, M. thyreoarytaenoideus. *Ph. L.*, Pharynx lumen; *S. N. S.*, sympathetic nervous system; *thy. c.*, thyreoid cartilage; *n. XII*, n. hypoglossus; *cri. lat.*, M. cricoarytaenoideus lateralis; *thy. ary.*, M. thyreoarytaenoideus.

Fig. 3 Frontal section of human Embryo no. 317 (12.5 mm.) to show epiglottis thyreoid cartilage, and hyoid bone. Ant. Card., anterior cardinal vein.

Fig. 4 Frontal section of human Embryo no. 317 (12.5 mm.) to show superior laryngeal nerve. *sup. lary.*, superior laryngeal nerve; *car. a.*, carotid artery. (The cricoarytaenoideus lateralis mass (*cri. lat.*) includes whatever there is of the thyreoarytaenoideus muscle.)

Fig. 5 Frontal section of human Embryo no. 317 (12.5 mm.) to show M. cricothyreoideus and arytaenoid masses. *Ary. m.*, arytaenoid masses; *cri. thy.*, M. cricothyreoideus.

Fig. 6 Frontal section of human Embryo no. 317 (12.5 mm.) to show M. cricoarytaenoideus posterior, interarytaenoideus and nerve recurrens. *tr.*, trachea; *int. ary.*, M. interarytaenoideus.

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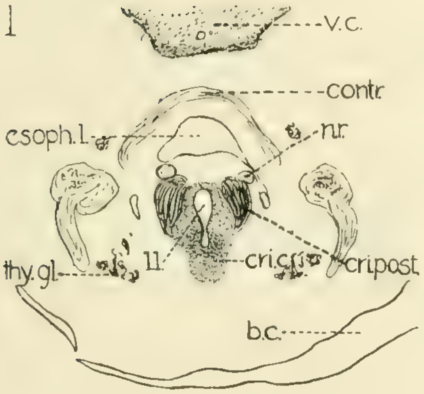
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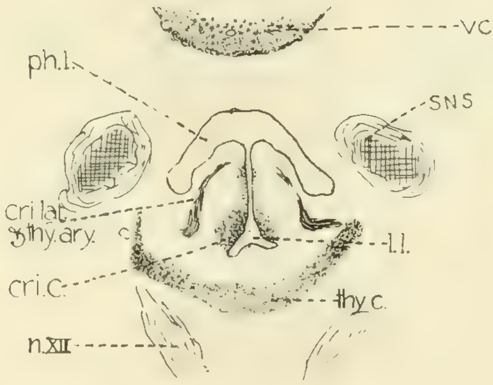
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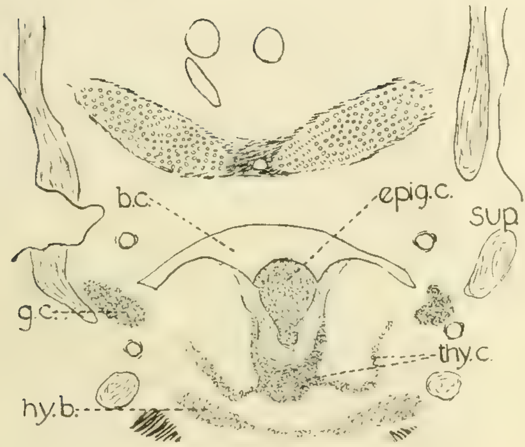
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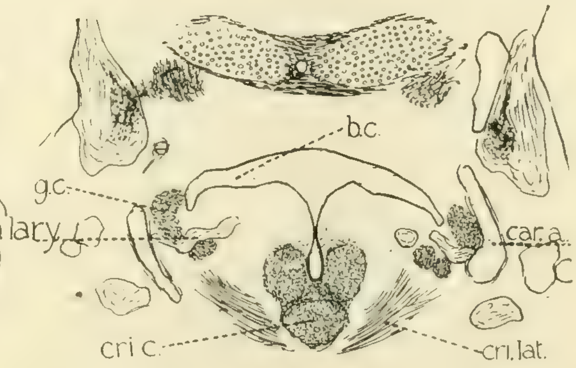
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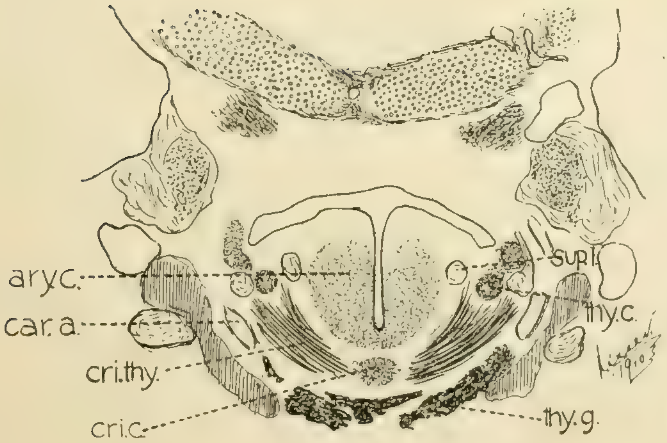
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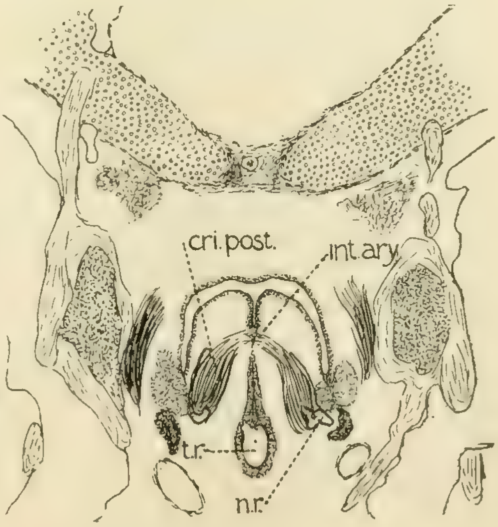
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and assume a clearer outline. Figs. 4 and 5, show two sections of this cartilage. These drawings of course, are of necessity, exaggerated, as no such absolutely isolated areas exist for they fade off imperceptibly into the surrounding tissue, and outlines must be somewhat arbitrarily decided upon. The lumen is still narrow and slit-like, so that the circular, ring-like appearance of the cricoid is not yet established. The sides seem compressed more or less one upon the other.

The artaenoid masses, (fig. 4), make their appearance at this time, and although rather intimately related to the cricoid mass, nevertheless permit of recognition. They are roughly of oval shape and bear little resemblance to their adult appearance. They are of course composed purely of precartilage; they develop more slowly than do the cricoid or thyreoid, but keep abreast of the epiglottis in their growth. Even at the 20 mm. stage when the cricoid and thyreoid show a predominance of chondrification, the arytaenoids and epiglottis are still represented only by condensed mesenchyma.

The epiglottis. The epiglottis is shown in fig. 3; it is situated at a lower level than in the adult.

There is a crowding together of laryngeal structures at this period in development. In the adult the length or height of the epiglottis is much greater than its breadth while in the embryo the length and breadth are about equal. This congestion of the cartilage is very likely due, in great part, to the general ventral curvature of the entire embryo, especially the way the head is bent upon the body and with the subsequent lengthening out and straightening out of the whole body, the laryngeal cartilages naturally assume their adult relationship. Such changes, though partial, have already occurred by the 20 mm. stage, as seen in fig. 38 of the model and illustrate the tendency, which is fulfilled more markedly later.

The hyoid bone and styloid process are composed of condensed mesenchyma and are very clearly outlined. Fig. 3 shows this. The greater cornu is developing rapidly and has attained large proportions.

THE MUSCLES

The figs. 4 and 5 which are intended to illustrate, among other things, some of the musculature of the larynx, may give a wrong impression, which possibility therefore, I hasten to avoid. There is by no means the fibrillation in these muscles, as shown in fig. 6. The muscles, with the exception of the circoartyaenoideus posterior, which is faithfully drawn—are merely condensed mesenchyme, with the barest suggestions of fibrillation, and the character given them in the drawings are merely for the sake of clarity.

M. cricoarytaenoideus posterior is very well developed, as shown in fig. 8, of large size, and well advanced to fairly complete fibrillation. The muscle is somewhat more laterally situated than in the adult, but otherwise conforms closely to the later stages. It is plentifully innervated by the recurrent laryngeal nerve.

M. cricoarytaenoideus lateralis (fig. 4) though not nearly so precisely outlined, this muscle mass, nevertheless, assumes considerable proportions at this stage. It is difficult, as it was in the case of the 10.5 mm. embryo, to decide whether the premuscle tissue represents only the cricoarytaenoideus lateralis, or whether it also includes what is to become the thyreoarytaenoideus m. No fibers originating from the mesial surface of the lateral portions of the thyreoid cartilage were found, not even a slight trend of the condensed mesenchyma. So that the muscle may not appear at all till later in development. The thyreoepyglotticus and aryepiglotticus are both absent.

The interartyaenoideus (fig. 6) has made its appearance, but is partly attached to the insertion fibers of the circoarytaenoideus posterior. It is fibrillated.

The cricothyreoideus muscle (fig. 5) can be made out at this time, sending its fibers from the ventrolateral portion of the cricoid to the mesial surface of the thyreoid. It cannot be entirely separated from the cricoarytaenoideus lateralis mass, but its innervation by the superior laryngeal nerve helps in the identification.

NERVES—(*Figs. 4, 5, 6*)

The n. laryngeus superior (fig. 4) and inferior (fig. 6) can be followed perfectly in these sections, from the point where they leave the vagus, to their ultimate endings in the various muscles. No reconstruction was made to prove positively an anastomosis between the two, although a study of the sections suggested this strongly. The innervations of the various muscle masses were definitely seen.

Embryo 144—14 mm. Sagittal sections—50μ

Both the embryo under consideration and the following one of 16 mm. were cut sagittally and this circumstance affords excellent opportunity for comparison. But the conclusion forced upon one is, that there is very little disparity between the two,—practically no changes of sufficient significance to warrant recording. This, of course, may be due to the fact that the shorter embryo, may be older than its length would indicate, or *vice versa*, as regards the longer embryo. Yet it is not unlikely that little progress is made in the various laryngeal structures during this period of development. There is an indication of beginning chondrification in the 16 mm. embryo, whereas there is none whatsoever in the 14 mm. embryo, but otherwise the differences are negligible. A few drawings were included of some sections (figs. 7, 8, 9), in the 14 mm. embryo, which happen to show the musculature a little more distinctly than in the 15 mm. sections—and they will also serve the purpose of visualizing the similarity mentioned above. Accordingly, a detailed description of the larynx in this embryo will be omitted, as the description of the 16 mm. one will suffice. And moreover, more complete studies, such as graphic reconstructions, were made on the older embryo.

Embryo 43-16 mm. Sagittal sections

Embryo 43 measures 16 mm. V.B. and 4 mm. N.B. about six weeks old.

THE CARTILAGES

The thyreoid cartilage is a peculiar structure at this stage, still consisting purely of condensed mesenchyma. The lateral alae are united ventrally, but it is to the odd shape of the lateral masses, that I would call attention. Fig. 13 shows a graphic reconstruction of this cartilage from the side view. The superior cornu, fig. 13, is in evidence, and is in correct relation to the greater cornu of the hyoid bone, between which develops the thyreohyoid ligament. Another point in favor of this being the superior cornu of the thyreoid, is the attachment to it of the inferior constrictor pharyngis, as seen in fig. 11. Posterior, and below them, protrudes a curious cylindrical mass of condensed mesenchyma, which undoubtedly forms the rudiment of the inferior cornu of the thyreoid. It overlaps the cricoid and is in close apposition to it as shown in fig. 14. Probably there is no actual articular facet at this stage. Anteriorly, there is a strange projection, without apparent attachment to anything ; it seems to be evidence of greater activity of growth in the ventral part of this lateral mass, just as the superior and inferior cornua are the results of active growth in the posterior portion of this lateral mass. Apparently then, the directly lateral part lags behind temporarily, and the peculiar gap between the anterior cornu (as I call this odd projection) and the superior cornu, is filled in during the next week or so.

In the 20 mm. stage, there appears to be a slight tendency to condensation in this area, not marked enough to be included in the reconstruction of this stage.

The cricoid cartilage (fig. 10) consists of pure condensed mesenchyma, with no evidence as yet of chondrification. Although rather crude in outline, yet it begins to suggest roughly the maturer form. Its relation to the thyreoid cartilage resembles the adult rather closely, and the continued ring, ventral and dorsal, is now complete. Also, the posterior portion is enlarging and begins to show advances over the relatively slower growth of the anterior arcus, conforming with the adult type. Certainly, it is further advanced than the thyreoid cartilage.

Fig. 7 Sagittal section of human Embryo no. 144 (14 mm.) to show, especially, M. interarytaenoideus.

Fig. 8 Sagittal section of human Embryo no. 144 (14 mm.) to show hyoid bone and thyreoid cartilage, M. cricothyreoideus, and tongue region. *thy. hy.*, M. thyreohyoideus; *omo. hy.*, M. omohyoideus; *mylo. hy.*, M. mylohyoideus; *sterno. thy.*, M. sternothyreoideus; *sterno. hy.*, M. sternohyoideus· *dig.*, M. digastricus; *gen. gl.*, M. genioglossus; *X*, N. vagus.

Fig. 9 Sagittal section of human Embryo no. 144 (14 mm.) to show M. Cricoarytaenoideus lateralis, thyreoarytaenoideus, cricoarytaenoideus posterior, and nerve recurrens. *ary. c.*, arytaenoid cartilage; *esoph.* m., esophageal musculature; *gen. hy.*, M. geniohyoideus.

Fig. 10 Graphic reconstruction of cricoid and arytaenoid cartilages in human Embryo no. 43 (16 mm.).

Fig. 11 Graphic reconstructions of pharyngeal constrictors in human Embryo no. 43 (16 mm.). *m. constr.*, middle constrictor muscle; *inf. constr.*, inferior constrictor muscle.

Fig. 12 Graphic reconstruction of laryngeal musculature in human Embryo no. 43 (16 mm.). (Thyreoid cartilage partly removed).

Fig. 13 Graphic reconstruction of thyreoid cartilage hyoid bone, and styloid process in human Embryo no. 43 (16 mm.).

Fig. 14 Graphic reconstructions of larynx cartilages in human Embryo no. 43 (16 mm.). *sup. c.*, superior cornu (thyreoid cart.); *inf. c.*, inferior cornu (thyreoid cart.).

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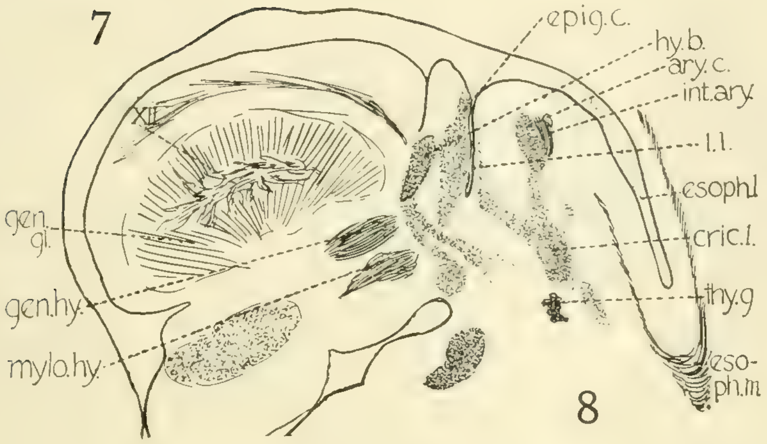

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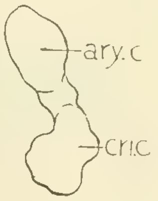

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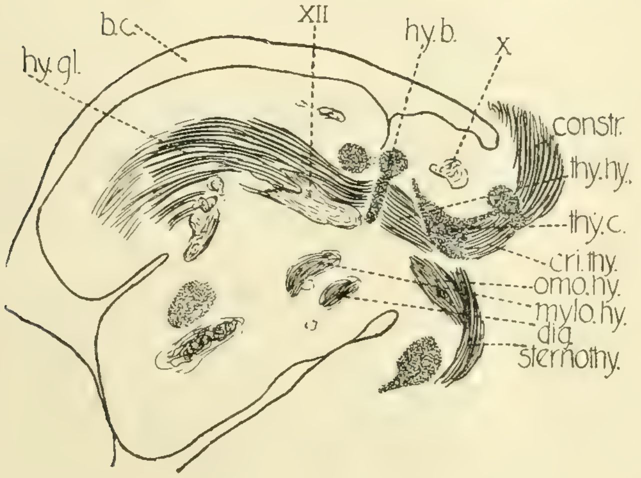

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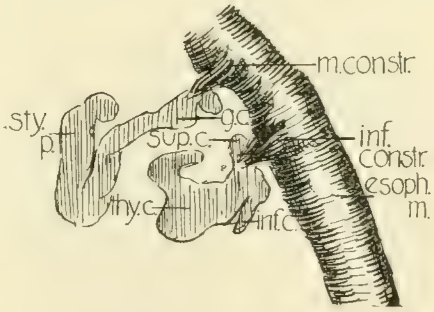

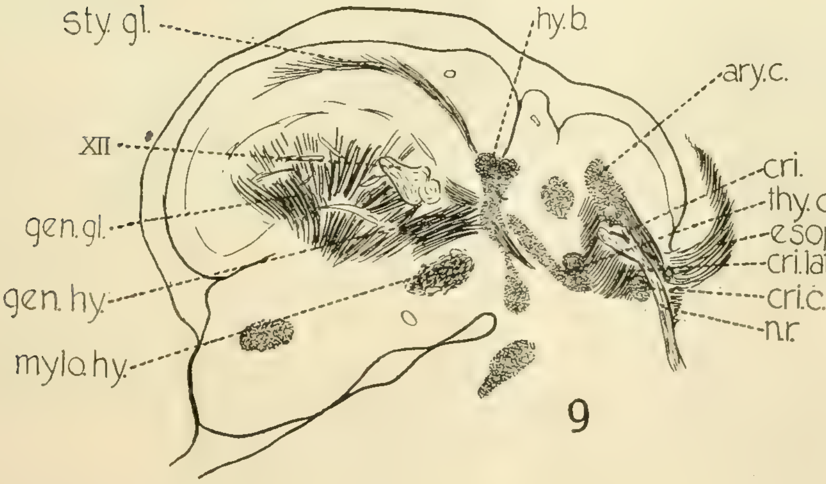

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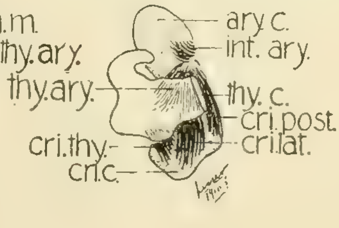

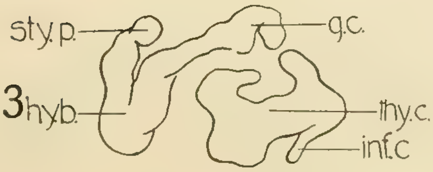

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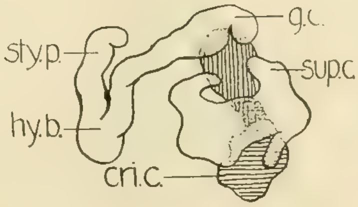

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Fig. 15 Sagittal section of human Embryo no. 43 (16 mm.) to show laryngeal musculature and nerve recurrens. *Meck. c.*, Meckel's cartilage.

Fig. 16 Sagittal section of human Embryo no. 43 (16 mm.) laryngeal region. *s. m. gl.*, submaxillary gland.

Fig. 17 Graphic reconstruction of 9th, 10th, and 12th cranial nerves in larynx region of human Embryo no. 43 (16 mm.). *ana.*, anastomosis between superior laryngeal and inferior laryngeal nerves; *m br.*, motor branch of superior laryngeal nerve.

Fig. 18 Sagittal section of human Embryo no. 43 (16 mm.) laryngeal region.

Fig. 19 Sagittal section of human Embryo no. 43 (16 mm.) laryngeal region. *s. m. gangl.*, submaxillary ganglion.

Fig. 20 Sagittal section of human Embryo no. 43 (16 mm.) laryngeal region.

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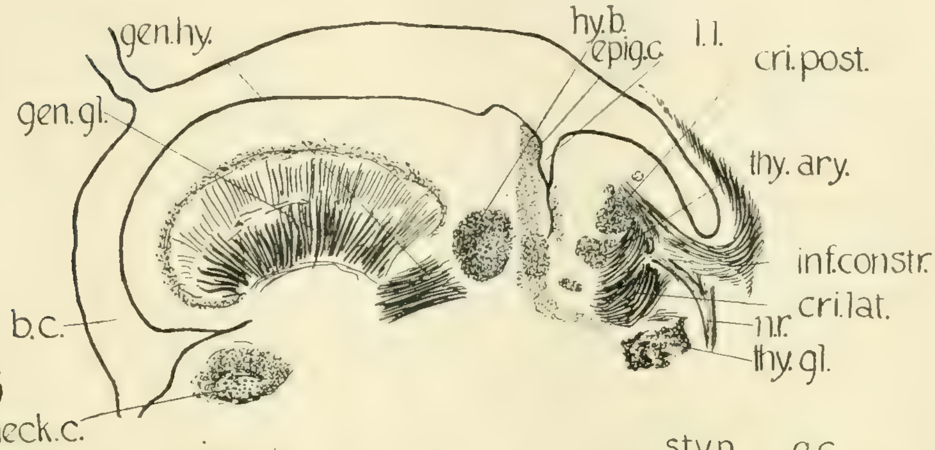

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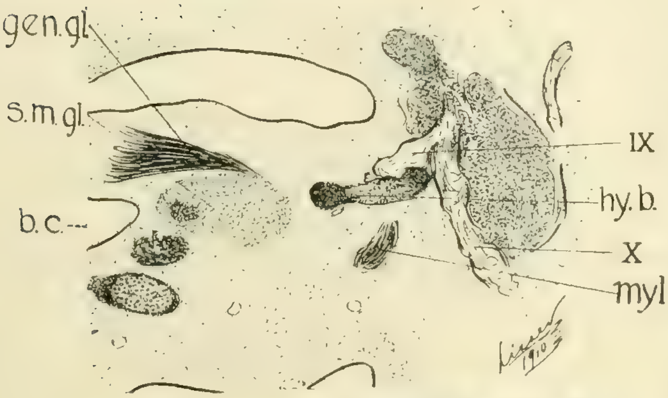

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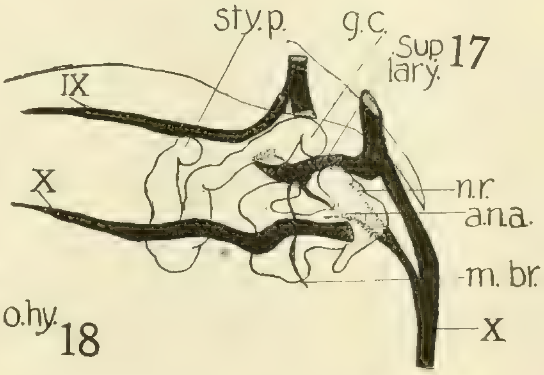

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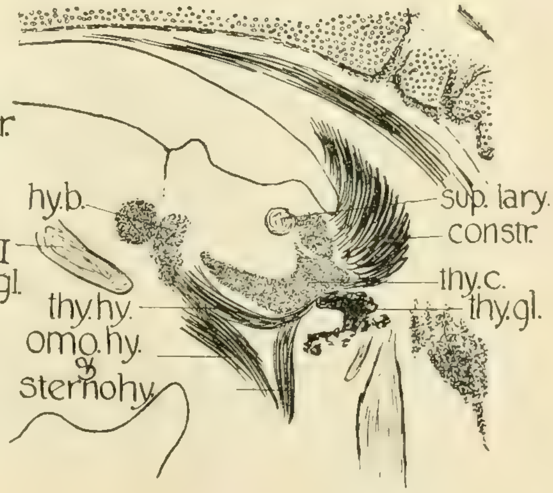

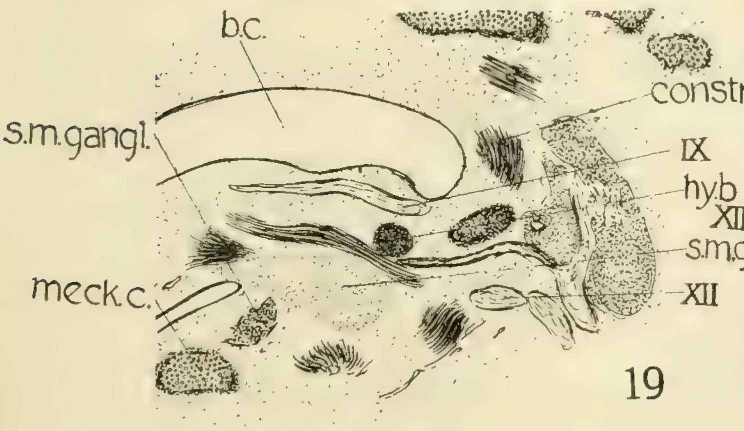

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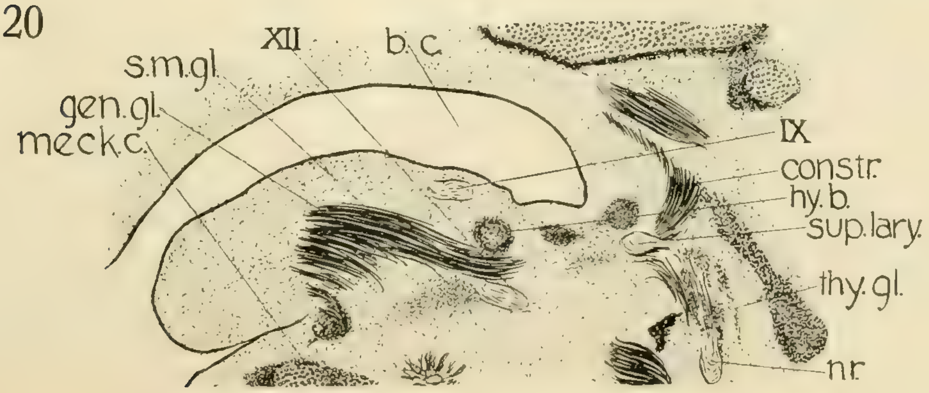

The arytaenoids are still represented by a roughly oval mass of condensed mesenchyma, with no accuracy of form or outline.

The arytaenoids are still represented by a roughly oval mass of condensed mesenchyma, with no accuracy of form or outline.

Fig. 10 is a reconstruction of one of them from a lateral view. The mass is continuous with the cricoid mass, as indicated by the cross lined area, (fig. 14) but its main arytaenoid portion is stained deeply enough to differentiate it absolutely from being a part of the cricoid mass.

The epiglottis shows but little advance over its condition in the earlier embryos, except for some gain in length over breadth, but the mass out of which it assumes its adult shape, is easily recognized.

The hyoid bone and styloid process begin to show small areas of chondrification, and their appearance is seen in a reconstruction fig. 13 and 14. The attachment of the middle constrictor to the greater cornu is shown in fig. 11.

THE MUSCLES

In a 14 mm. embryo Strazza says that a muscle mass, of spindle shaped formation can be made out laterally in cross sections, but that no distinct muscles can be isolated at this stage, and that the mass simply represents laryngeal musculature. And at 16 mm. he distinguishes a muscle band, bending around with the posterior convexity, which in its upper portions he calls the arytaenoideus transversus (interarytaenoideus), but considers this band a continuous muscle mass, only differentiated later by the development of the cartilages. Further down he thinks it to be the cricoarytaenoideus lateralis and thyreoarytaenoideus, but says it is continuous with the above, only spread over a greater area. So he calls the larynx musculature an arch, which however, is not entirely horizontal, but goes from above and behind, to below and in front. Now it is true that considerable interlacing of fibres exists at this stage, but not much more than occurs in careful dissections of the adult larynx. It is also true that further separation and development of the cartilages will bring about clearer differentiation of the muscles. But that a continuous muscle band, which cannot be differentiated into individual muscles, exists at this stage is not in accord with the results shown, especially in fig. 12, a reconstruction of the 16 mm. stage, and in figs. 2, 8, 9, faithful drawings of the sections of the 14 mm. embryo, and fig. 15 of the 16 mm. embryo.

The cricothyreoideus, the cricoarytaenoideus posterior, and the interarytaenoideus are definitely isolated, while the cricoarytaenoideus lateralis and thyreoarytaenoideus are clearly separated from the others and are about as much separated from each other as they are in the adult. There is no need of describing them any further. The figures show them all in sufficient detail.

The constrictor muscles of the pharynx and œsophagus have been reconstructed and the middle and inferior constrictors stand out rather clearly. Nicolas states that the pharynx musculature only unites from two independent lateral halves at 3 cm. I have found perfect continuity at 10.5 mm. in the lower pharyngeal portion, more union at 12.5 mm. and complete union at 14 mm.

Several of the tongue and pharynx muscles have been included in the illustrations, and it will be seen that they are very clearly isolated and well developed even at the 14 mm. stage (figs. 7-9).

For the identity of these muscles, I am under obligations to Dr. Lewis, who very kindly gave me his own sketches, from which figs. 7, 8 and 9 were developed.

THE NERVES

The nerves, are reconstructed in fig. 14 and in addition to showing the superior laryngeus and n. recurrens and n. laryngeus inferior, which can be followed to their respective innervations and to their anastomosis, there is included the relations of these, to the glossopharyngeal and to the hypoglossus (also figs. 15, 16, 19, 20).

Embryo 22—20 mm. Transverse sections

Embryo 22 measures 20 mm. V. B. and 18 mm. N. B., about 7½ weeks old.

Fig. 21 Frontal section of human Embryo no. 128 (19.5 mm.) to show thyreoid cartilage and M. cricoarytaenoideus lateralis. *occ. b.*, occipital bone.

Fig. 22 Frontal section of human Embryo no. 128 (19.5 mm.) laryngeal muscles and cartilages.

Fig. 23 Frontal section of human Embryo no. 128 (19.5 mm.) to show cricoid cartilage, M. cricoarytaenoideus posterior, and thyreoid gland.

Fig. 24 Graphic reconstruction of cricoid cartilage, posterior veiw, in human Embryo no. 22 (20 mm.) *3*, anterior arch; *4*, posterior arch.

Fig. 25 Graphic reconstruction of cricoid cartilage, lateral veiw in human Embryo no. 22 (20 mm.). *1*, articular facet for thyreoid cartilage; *2*, articular facet for arytaenoid cartilage.

Fig. 26 Same as fig. 25, showing extent of choudrification. *tr. c.*, true cartilage; *pre. c.*, pre-cartilage (condensed mesenchyma).

Fig. 27 Graphic reconstruction thyreoid cartilage in human Embryo no. 22 (20 mm.) showing extent of chodrification.

Fig. 28 Graphic reconstruction of laryngeal cartilages in human Embryo no. 22 20 mm.) *thy. hy., 1.* thyreohyoid ligament.

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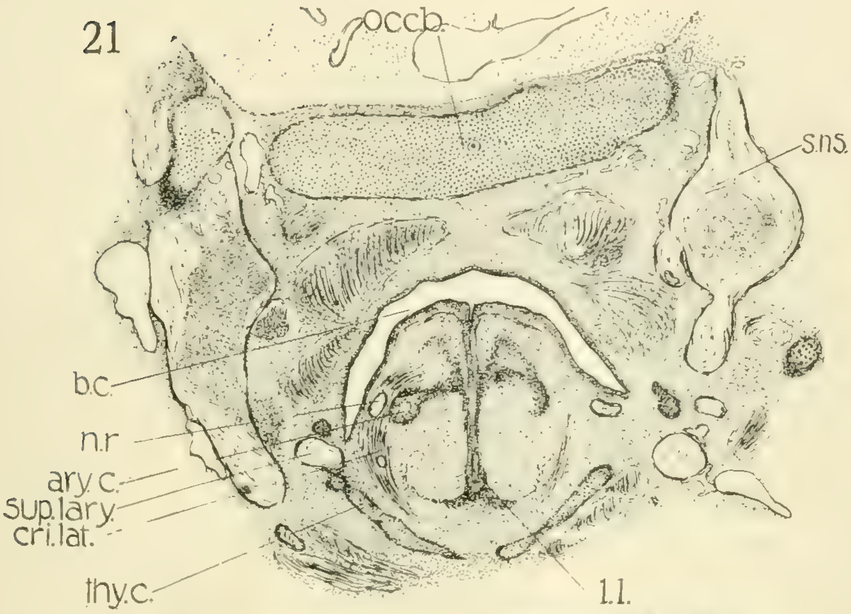

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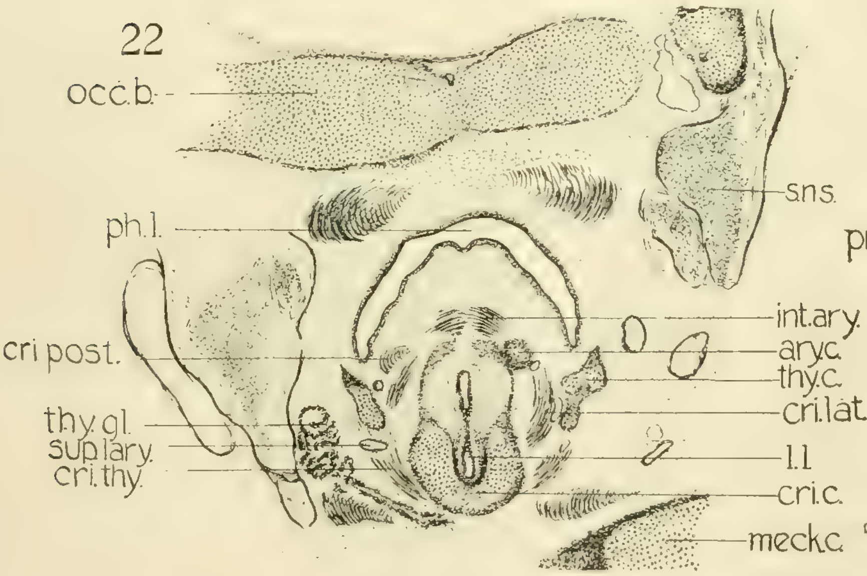

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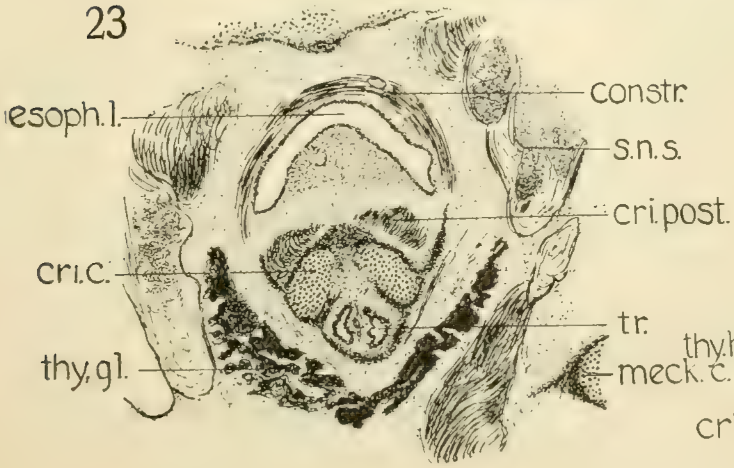

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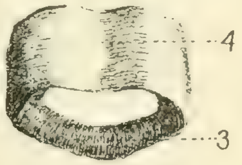

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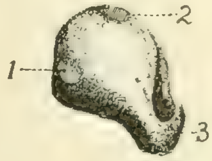

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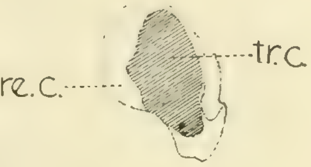

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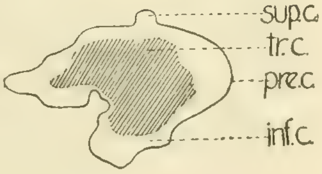

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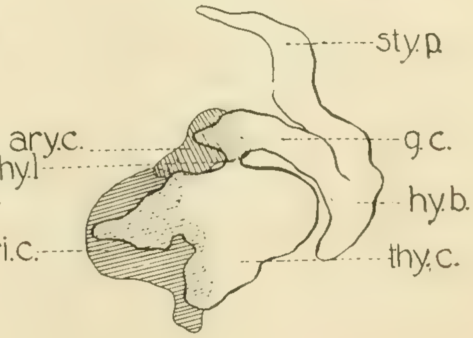

THE CARTILAGES

Thyreoid. This cartilage is easily recognized at this stage and has a definite outline. Although it has not attained the adult shape, in all particulars, it has, nevertheless, developed into the adult type. Not all of the tissue representing this structure, is composed of true cartilage, and fig. 27 gives, from a lateral aspect, some conception of the relative portions of chondrification and precartilage. Very little, if any, of the anterior, ventral, median portion, between the two wings is represented by cartilage, this being almost entirely condensed mesenchyme. This is well illustrated in figs. 29 and 30, which likewise depict the broad generous curve (convex throughout) uniting the two wings ventrally. The notched appearance with the prominent ventral ridge is not evident as yet. This ventral portion, in a vertical direction (caudocephalad) is not very extensive, being appreciably smaller in height than in the adult (relatively).

The wings of the thyreoid cartilage are quite well developed indeed, as can be seen in figs. 29 and 32, showing considerable chondrification.

The upper cornu is prominent, but it does not present the upward curve, as strikingly as in the adult. Its attachment to the greater cornu of the hyoid by the thyrohyoid ligament is well seen,—the latter appearing as condensed mesenchyme. Just where the thyreoid leaves off, and the ligament begins, and where the latter establishes connection with the hyoid, cannot be definitely ascertained, but the continuity of the parts is easily demonstrated, by graphic reconstruction, as shown in fig. 28.

The inferior cornu of the thryeoid is of a more exaggerated type than in the adult, projecting from the wings over the lateral posterior portion of the cricoid. The relations of these two cartilages are shown in fig. 28. A study, in cross sections, of these two cartilages reveals an unmistakable apposition—not approximate, but in close contact. Whether there is an actual articular facet present at this stage, is difficult to determine. The wax model suggests such a possibility very strongly but the appearance is within the limits of error, unavoidable in the construction of such a model. Nicolas ('94), in his excellent studies on the thyreoid cartilage, insists that the articulations crico-thyreoid and cricoarytaenoid appear very late. I am inclined to disagree with him on that score. Certainly the rudiments of such an articulation are present, at least of the crico-thyreoid. However I would not make a positive assertion as to the completeness of the articulation, because of the possibilities of error as mentioned above.

The cricoid (fig. 25). This structure is likewise very well developed at this stage, corresponding with the adult type even more closely than does the thyreoid. Its seal ring appearance is quite characteristic. Its shape is almost round and accordingly shows a corresponding development in the larynx lumen,—for the two are interrelated in their growth. Fig. 26 illustrates the extent of chondrification in this cartilage. The ventral arcus is, however, condensed mesenchyme (pre-cartilage). It is quite definite, low in front, gradually higher behind to the posterior arcus, which is very large. The latter's posterior surface seems to possess already the two flat fossæ for the origin of the mm. crico-arytenoidei posteriores. Again, there is typical overlapping of the arytaenoid cartilage over the cricoid, in its superior portion, and the typical apposition again strongly pictures the possibility of a definite articulation. The relation of the two cartilages is seen in fig. 28.

The arytaenoids. These cartilages are rather behind the thyreoid and cricoid in development, certainly as regards chondrification, which has scarcely begun, as they are almost entirely composed of condensed mesenchyme. Their shape approaches that of the adult type—but the fovea triangularis and fovea oblonga on the ventral surface are not at all clear. There is no definite processus muscularis, but the presence of all the muscles in characteristic position, and with characteristic attachment, indicates no doubt the place where this process will appear.

The cartilages of Wrisberg and Santorini, according to Nicolas, "do not appear until the other cartilages have taken definite form, not becoming cartilagenous until the epiglottis is fully formed at 6½ months." The first part of the statement is rather indefinite. I should say that all the other cartilages had definite form even before this stage, but surely at the 20 mm. stage. Certainly, the

Fig. 29 Cross section of human Embryo no. 22 (20 mm.) to show especially superior laryngeal nerve and nerve recurrens.

Fig. 30 Cross section of human Embryo no. 22 (20 mm.) to show, especially, M. interarytaenoideus and aryepiglotticus. This section shows at the mark (x) the tendency to continuity between the laryngeal and pharyngeal musculature, as mentioned by Strazza.

Fig. 31 Cross section of human Embryo no. 22 (20 mm.) (very low in laryngeal region).

Fig. 32 Cross section of human Embryo no. 22 (20 mm.) to show thyreoid cartilage, cricoid cartilage, and hyoid bone. Also M's cricoarytaenoideus posterior and thyreorarytaenoideus.

Fig. 33 Graphic reconstruction of nerve recurrens and its branches in relation to the laryngeal muscles and cartilages in human Embryo no. 22 (20 mm.) *br. 1*, Branch of n. recurrens, to M. cricoarytaenoideus posterior; *br. 2*, branch on n. recurrens to M.'s cricoarytaenoideus lateralis and thyreoarytaenoideus; *br. 3*, branch of n. recurrens to M. thyreoepiglotticus; *br. 4*, branch of n. recurrens to M.'s inter arytaenoideus and aryepiglotticus.

Fig. 34 Graphic reconstruction of motor branch of superior laryngeal nerve in human Embryo no. 22 (20 mm.).

Fig. 35 Graphic reconstructions of laryngeal muscles, and cartilages, and their relations in human Embryo no. 22 (20 mm.) *ary. epi.*, M. aryepiglotticus; *thy. epi.*, M. thyreoepiglotticus; *c. s.*, *thy. c.*, cut surface thyreoid cartilage.

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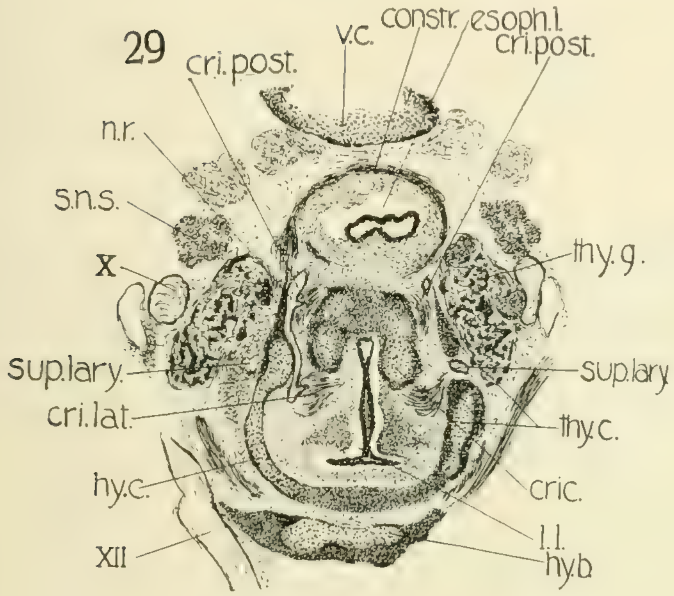

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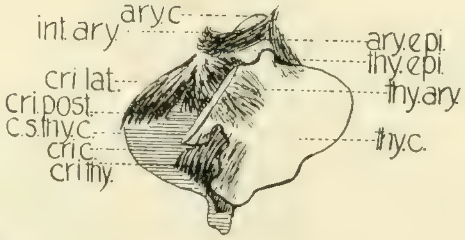

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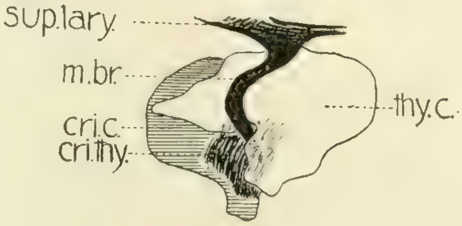

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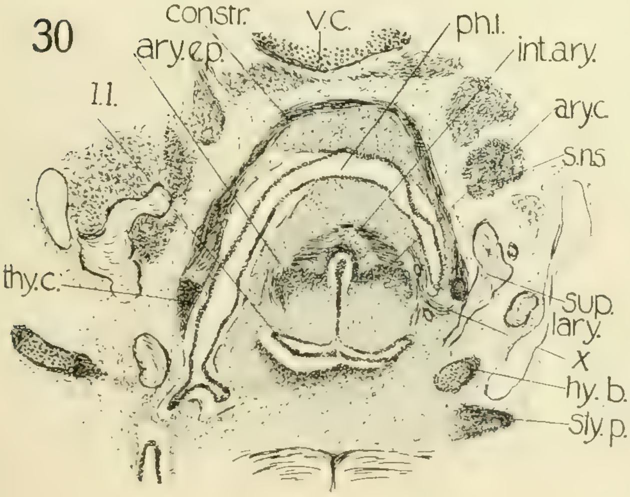

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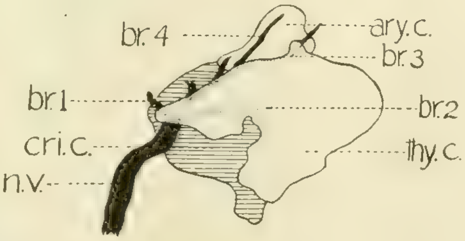

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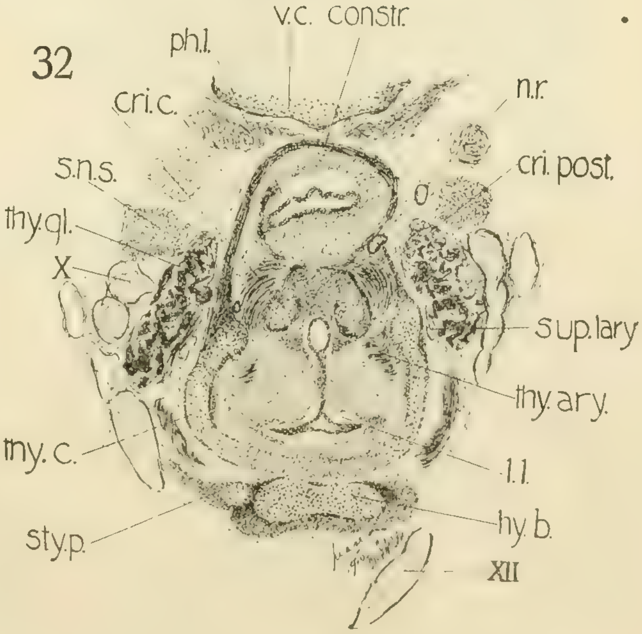

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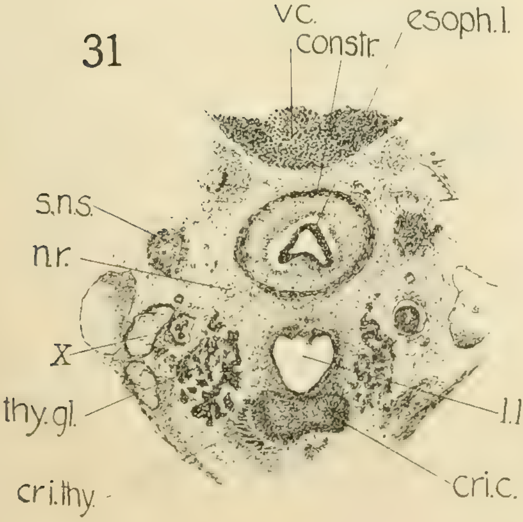

cartilages of Wrisberg and Santorini are not present at this period as definite separate structures, but there is a suggestion of the latter in the smaller model of this stage—an appendage of the arytaenoids—fig. 36.

The Hyoid bone and styloid process need not be described in detail. A very good idea of them can be obtained from a study of figs. 28, 29 and 32. The drawings of the model, figs. 37, 38 and 39, show the general shape, and relation to contiguous structures, and the section drawings give some idea of the extent of chondrification.

THE MUSCLES

At this stage the musculature is quite distinct and all the muscles permit of clear differentiation. Fibrillation is fairly extensive, especially in the cricothyreoideus and cricoarytaenoideus posterior. The muscles have attained their adult relationship and reconstructions reveal their character with considerable precision. Thus I cannot agree with Kanthack ('92), who says that at four months the cricothyreoid and constrictor are in close contact and cannot be separated one from the other; that it is impossible at two months to separate the interarytaenoideus and cricoarytaenoideus posterior. That up to the fourth month the fibres have the same direction and that the muscles cannot be really differentiated until the fourth month. He adds that it is impossible to separate the thyreoarytaenoideus and cricoarytaenoideus lateralis, which holds also for the child and adult. Although 'impossible' is expressing it a bit strongly yet the latter part of the statement is well taken, as even in the child and adult the differentiation is not always clearly manifest.

The m. cricothyreoideus. Figs. 29 and 31 picture this muscle in section, and figs. 34 and 38 give the appearance in reconstruction, both wax models and graphic representation. Its origin on the external surface of the arcus cartilaginis cricoideae is as in the adult. It inserts on the medial surface of the inferior margin of the lamina cartilagins thyreoideae. It is a well developed muscle with numerous evidences of fibrillations. However there is no division into a pars recta and pars obliqua as in the adult, nor

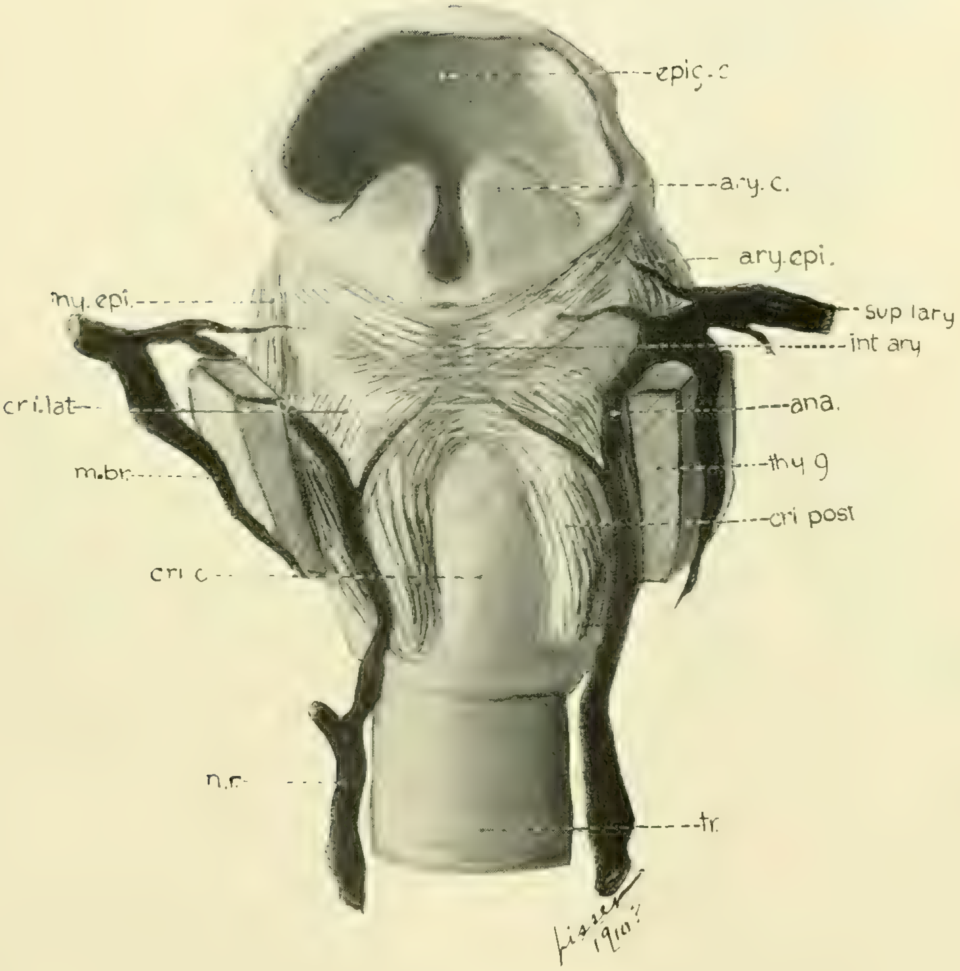

Fig. 36 Wax model of laryngeal muscles and nerves in human Embryo no. 22 (20 mm.). Drawn from the posterior aspect; the thyreoid cartilage has been partly removed above and posteriorly to admit of clearer view of muscles and nerves.

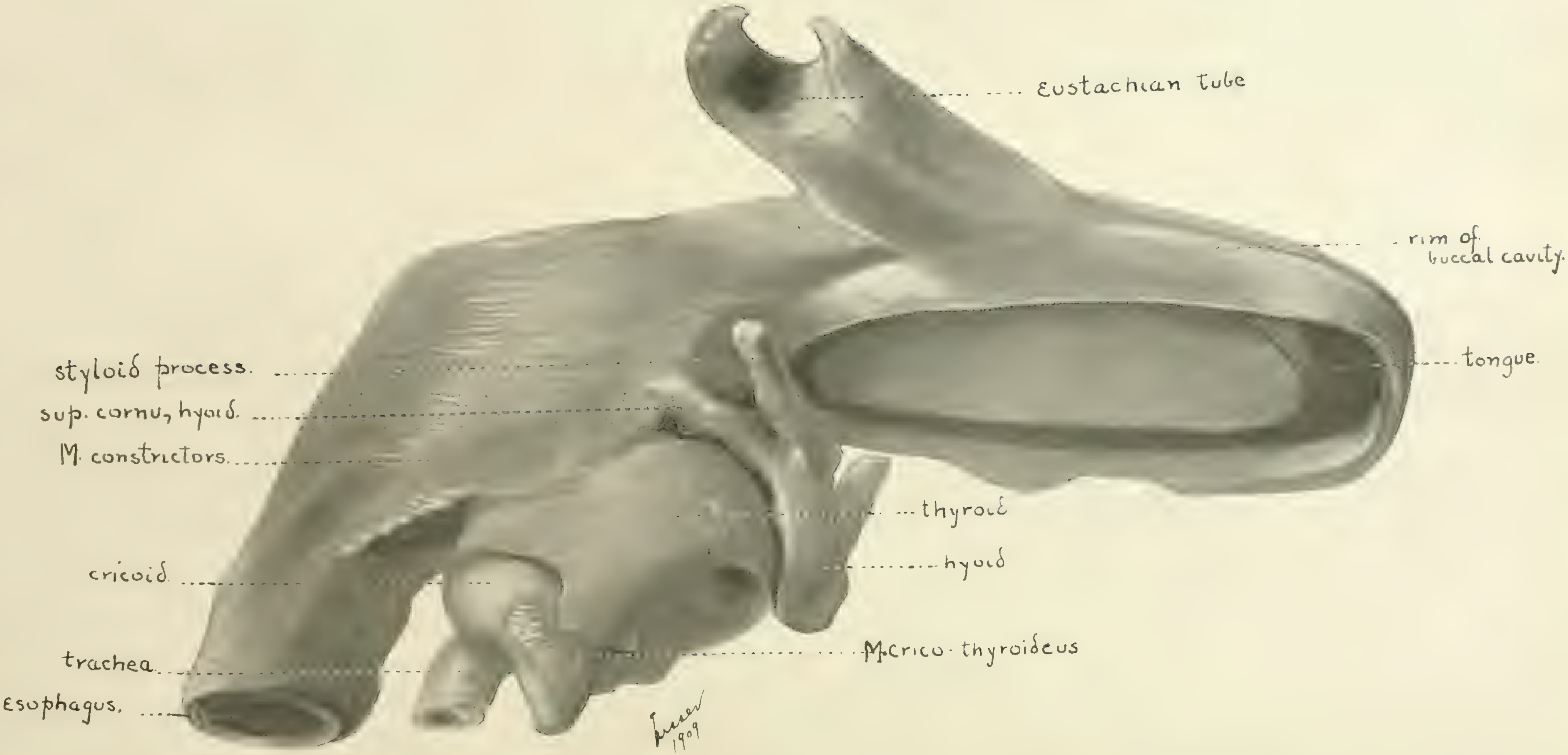

Fig. 37 Wax model of laryngeal region in human Embryo no. 22 (20 mm.). (Drawn from right side.)

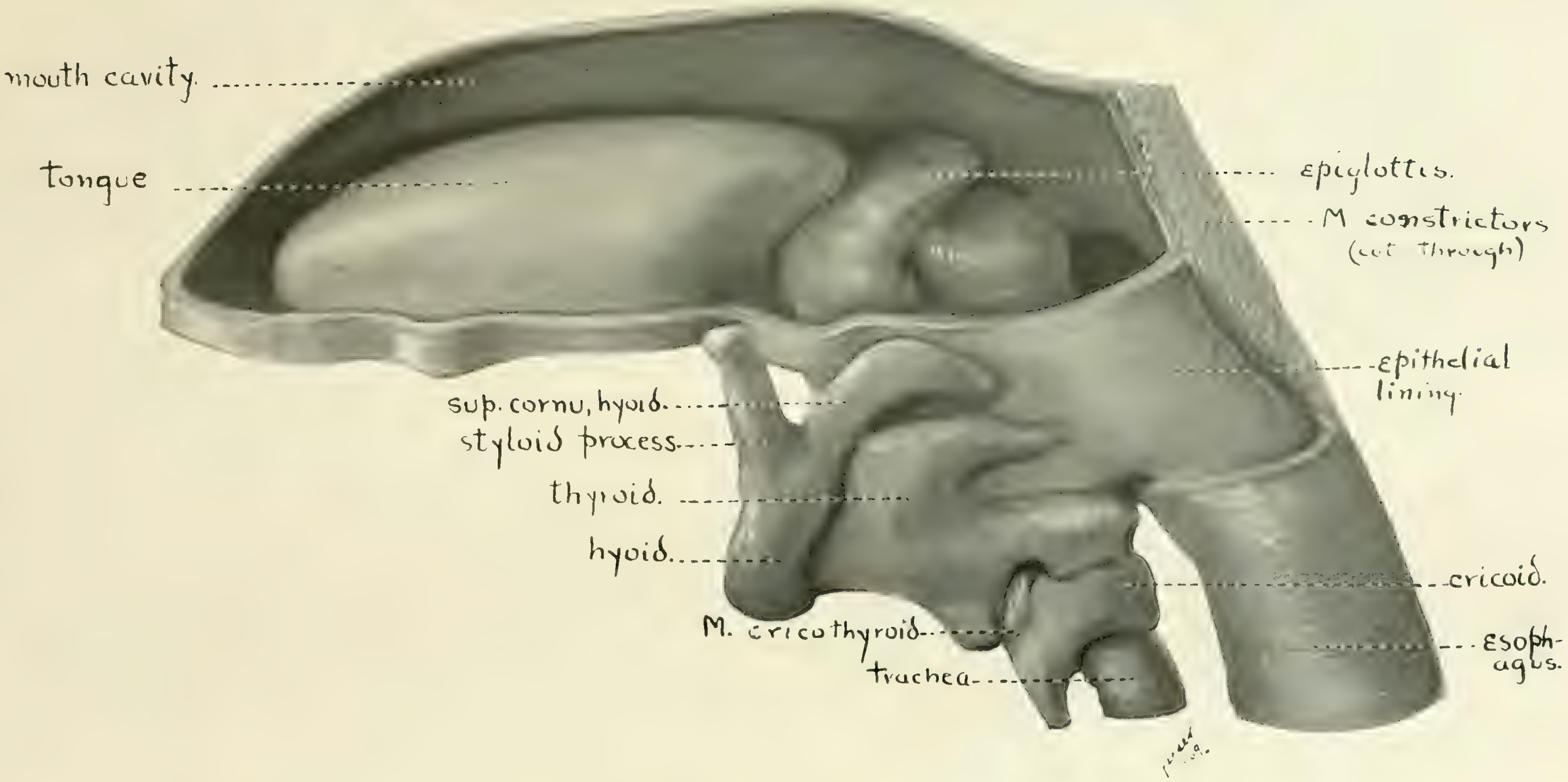

Fig. 38 Wax model of laryngeal region in human Embryo no. 22 (20 mm.). (Drawn from left side.)

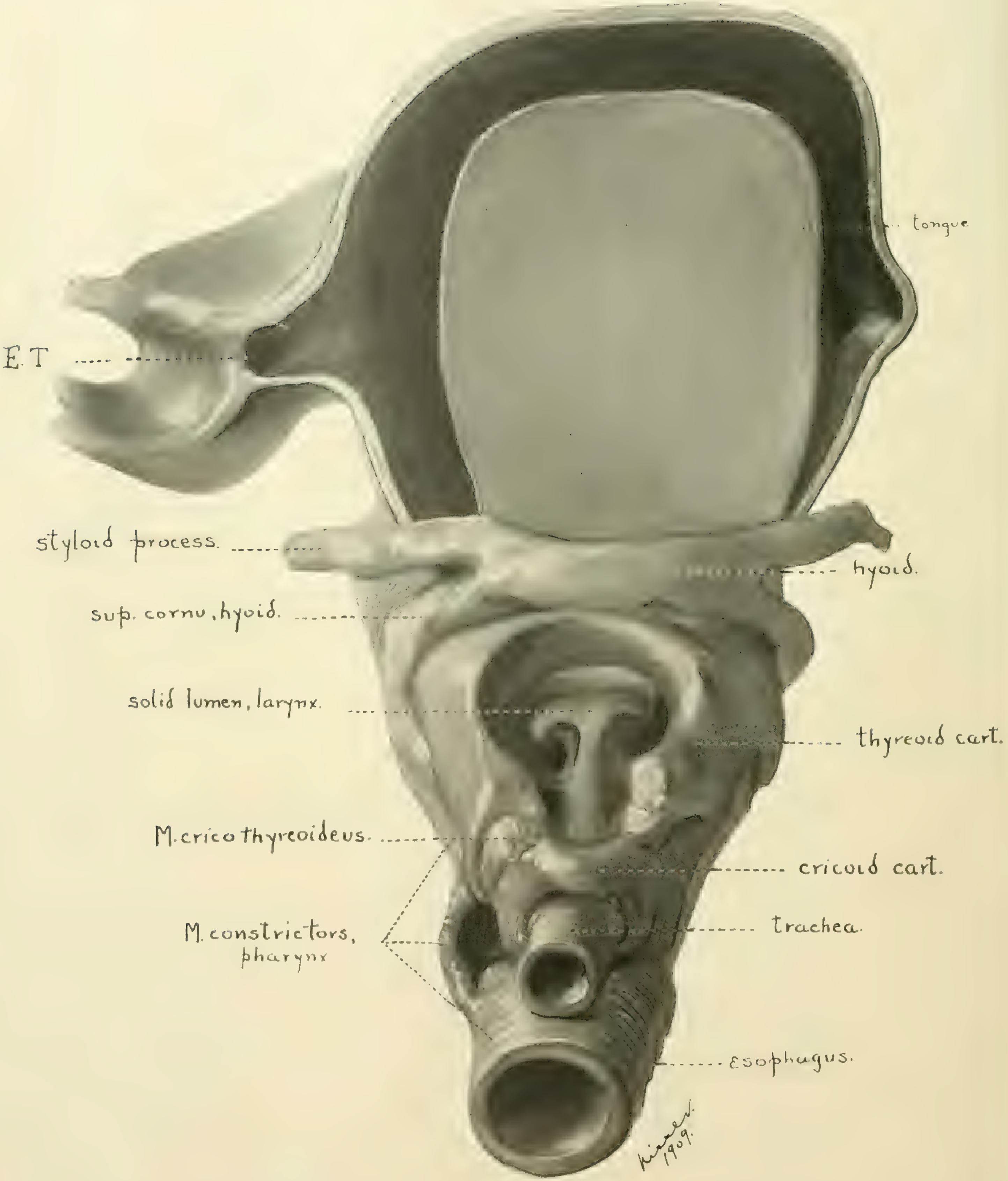

Fig. 39 Wax model of laryngeal region in human Embryo no. 22 (20 mm.). (Drawn from below)

does the muscle extend as far posteriorly in its insertion on the thyreoid. So that it would appear that the pars obliqua is a later manifestation, and owes its development to a more complete growth along the inferior cornu, along which it inserts more extensively later.

The m. cricoarytaenoideus posterior is a large conspicuous muscle as seen in fig. 36 of the wax model. It is in its adult position, arising from the medial inferior part of the lamina cartilaginis cricoideae and converging upward to insert on the apex of the processus muscularis cartilaginis arytaenoideae. It is closely applied to the cricoid cartilage as can be seen from a study of the sections and extends somewhat more lateralward on the cricoid cartilage than in the adult. Otherwise it conforms perfectly with the adult type.

The m. cricoarytaenoideus lateralis is a considerable muscle mass on the lateral surface of the cricoid cartilage, which from a study of the sections alone, would seem to merge in with the mass that by nature of position and relation should become the thyreoarytaenoideus muscle, but careful graphic reconstruction reveals the picture seen in fig. 35 where the independence of the muscle is plainly evident. Its fibrillation is not as extensive as that of the cricoarytaenoideus posterior. Its origin on the upper lateral surface of the cricoid and its insertion on the processus muscularis cartilaginis arytaenoideae is well marked.

M. thyreoarytaenoideus is likewise well marked at this stage and fig. 35 shows its relation to the median surface of the thyreoid and its insertion on the processus muscularis cartilaginis arytaenoideae. Undoubtedly some of its fibres and those of the cricoarytaenoideus lateralis interlace, just as they do in the adult, but the origin of the majority of the fibres from the thyreoid cartilage is distinct enough to justify a clear differentiation of the two muscle masses. Fürbringer ('75), maintains that the secondary interlacement of these muscles as exists in adults becomes apparent at 48 mm., and does not believe that the sphincter formation of adults is related to the primary sphincter. I have not studied embryos later than this stage, so that I am in no position to question the accuracy of this statement; yet I can hardly see why it is necessary to suppose that the interlacement, which is surely present according to my findings in an embryo of 20 mm., should disappear and reappear again at 48 mm.; for surely no very radical changes occur in development, as regards the larynx, after 20 mm.

M. interarytaenoideus. The fibres of this muscle show up beautifully in cross sections, and are unmistakably present in abundance in this stage. The muscle is well defined and quite thick. Figs. 30 and 36 give an idea of it, both in section and reconstruction. I hardly see why there should have been any difficulty in separating it from the cricoarytaenoideus posterior or thyreoarytaenoideus as was reported by Kanthack. The fibres run in a different direction and reconstruction shows it quite as sharply defined as in the adult.

M. aryepiglotticus. (figs. 35 and 36.) There is some doubt in mind as to whether the fibres which seem to make up this muscle can be clearly isolated from the interarytaenoideus; whether perhaps, the adult appearance is not due to the later development upward of the epiglottica. Primarily no doubt, these two muscles originate as one mass and at this stage cannot as yet be definitely isolated. The fibres which make up the aryepiglotticus seem to be continuous with the interarytaenoideus, merely having extended upward along the lateral surface of the epiglottis.

M. thyreoepiglotticus. At this stage and in this embryo, there seems to be an indication of this muscle as brought out in the reconstruction, figs. 35 and 36. It seems reasonable to assume that this muscle is originally unrecognizable from the thyreoarytaenoideus, and is again dependent for its differentiation upon the development upward of the epiglottica, which draws these fibres away from the other muscle mass. This has apparently occurred at this stage.

The constrictors. The esophageal musculature is very well advanced by this time, and by nature of the attachments to the superior cornu of the thyreoid and the greater cornu of the hyoid bone, a middle and inferior constrictor mass may be recognized. Fibrillation[2] is extensive.

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[2] Following the idea of Dr. Lewis, I have used the word fibrillation, not with the view of indicating fibrillae, but with reference to the definite direction of the muscle fibres.

In comparison with muscular development elsewhere in the body, and even in the neighborhood of the larynx, it is noticed that the laryngeal musculature is backward, especially in the richness and thickness of fibrillation; but I think that a study of the various illustrations will justify the statement that in a 20 mm. human embryo sufficient differentiation has occurred to admit of definite recognition of all the extrinsic and intrinsic muscles of the larynx. Considerable emphasis has been laid by most authorities on the sphincter formation of the larynx and pharnyx musculature; the continuity of the two, one sphincter within another, as it were. Notably the excellent contributions of Strazzer, Fürbringer and Kanthack lay stress on this. The existence of such a structure, is suggested in the earlier stages, but that this sphincter formation and horizontal fibre arrangement is the predominate feature at the 20 mm. stage, is I feel, not true, but capable of explanation. A study of cross sections, certainly inclines to such an impression; and fig. 30 suggests this especially; but reconstructions at this period of development do not bear this out, nor do the study of frontal sections as in embryo 19.5. mm.; and these studies surely suggest that the muscles are too clearly differentiated at this time to exemplify a sphincter arrangement, except in the same sense as such a general structure exists in the adult. Of course reconstructions, expecially wax models are by no means infallible, and not nearly as accurate as, for instance, the injection method for the study of blood vascular development; but I do consider the results of these reconstructions, both wax and graphic, to be well inside the limits of error.

THE NERVES

N. laryngeus superior (figs. 33, 34, and 36) is very distinct, of considerable proportion, and easily made out. It divides into two portions, the *ramus externus* and *internus* at about the level of the superior cornu of the hyoid. Ramus externus is by no means small in calibre, as seen in figs. 34 and 36, it descends almost vertically upon the outside of the muscle constrictor pharyngis inferior to the m. cricothyreoideus, in whose substance it can be readily traced, as seen in the graphic reconstruction fig. 34. The ramus internus extends externally to the level of the thyrohyoid membrane, and together with the a. laryngea superior perforates this, and divides into several branches, the main mass however passing downward along the median surface of the thyreoid cartilage and over the lateral internal muscles of the larynx to anastomose with the n. laryngeus inferior of the n. recurrens. This anastomosis is perfectly demonstrated at this stage by the wax model reconstruction. Some authorities state that in the adult this ramus internus is not entirely sensory, but sends some motor twigs to the m. interarytaenoideus. Others do not mention this. I cannot satisfy myself absolutely on this point. The anastomosis between the n. laryngeus inferior and superior is a large one, and it is difficult to say where one nerve leaves off and the other begins, and accordingly what distribution belongs to each.

N. laryngeus inferior. The chief resultant portion of the n. recurrens ascends along the medial surface of the lateral lobe of the thyreoid gland, under and medial to the m. constrictor pharyngis inferior, to the level of the articulatio cricothyreoidea, where it divides into a lateral and posterior branch; the former anastomosing with the n. laryngeus superior (ramus internus) and innervating the mm. cricoarytaenoideus lateralis, thyreoarytaenoideus, aryepiglotticus, and thyreoepiglotticus; the latter innervating the cricoarytaenoideus posterior and interarytaenoideus (figs. 33 and 36).

Nicolas ('94) describes a ganglion on the superior laryngeal nerve after it enters through the thyrohyoid membrane at the level of the arytaenoid eminences. In a couple of sections I have noticed tissue somewhat suggestive of such a structure, but have not investigated the matter carefully enough to add any thing further.

Embryo No. 128, 19.5 mm. Frontal sections

The larynx shows about the same conditions found in the preceding 20 mm. embryo. It was selected for study, before wax reconstructions were undertaken, and it was thought that a study of the various laryngeal structures, when cut in a different plane, would shed further light on some of the more doubtful features.

The cartilages are developed to practically the same extent as in Embryo 22 (20 mm.) and an idea of their appearance in frontal section, can be obtained by reference to figs. 21, 22, 23. There are no differences in the cartilagenous growth of sufficient importance to warrant a detailed description. The high degree of chondrification can be noticed in figs. 21, 22, 23.

The nerves likewise offer no difficulty for study, and can be followed to their respective terminations. For further details reference is made to the results obtained from a study of Embryo 22 (20 mm.).

A word might be said about the muscles, since the cutting into frontal sections presents at least this feature of interest. The conception of the laryngeal musculature as a sphincter laryngeus, as Strazza expressed it; with a definite horizontal direction to its fibers, expecially emphasized by Kanthack, is not borne out at all by a study of this embryo. The muscles like the cricoarytaenoideus lateralis and thyreoarytaenoideus have a frontal direction to their fibers, as seen in fig. 21 and 22 and by no means any horizontal trend. Moreover the muscles are all clearly differentiated, as much so as they are in the adult, and give no indication whatsoever of being related or connected with the pharyngeal constrictors.

CONCLUSIONS

1. Towards the end of the fifth week (?) (10.5 mm.) of embryonic life, the integral structures of the larynx, under which I include the precartilage masses and the premuscles masses, first make their appearance. The laryngeal nerves can be recognized but have not yet entered the interior of the larynx, although the recurrent laryngeal has pushed upward as far as the lower portion of the cricoarytaenoideus muscle. The rudiments of the cricoid and thyreoid and epiglottic cartilages can be identified. The arytaenoids have not made their appearance. Four premuscles masses are present, two on each side of the larynx; all are inde-

pendent of each other. The upper group contains the crico-thyreoideus, cricoarytaenoideus lateralis and thyrearytaenoideus. The lower mass, the cricoarytaenoideus posterior is well differentiated. The interarytaenoideus has not yet appeared nor the aryepiglotticus nor the thyreoepiglotticus. There is apparently no relation with the pharyngeal constrictors.

2. During the following week, the sixth week of embryonic life (14 and 16 mm.) the cartilages though still existing purely as condensed mesenchyma, have developed into distinctly outlined masses; the arytaenoids have made their appearance during this time. The muscles have grown with considerable rapidity, so that by the end of the sixth week, they can be well differentiated from each other; have received all their innervation, and have developed abundant fibrillation. The thyreoepiglotticus and aryepiglotticus are still absent however. The nerves have advanced correspondingly and can be traced to their final terminations, and the anastomosis between the inferior and superior laryngeal nerves has been accomplished. In comparison to the adult form, the larynx at this stage gives an impression of being crowded upon itself.

3. By the end of the seventh week (?) (20 mm.) the larynx has assumed its adult relationships, both externally with regard to neighboring organs and internally with respect to its various constituents. All the cartilages with the exception of those of Wrisberg and Santorini are well developed. All the muscles are clearly represented, capable of absolute differentiation; and the gross nerve supply of the region is likewise complete.

4. The existence of such a sphincter as Strazza describes seems very doubtful indeed, as there are no clear indications of it in the specimens studied, and the very first indication of the laryngeal musculature shows several independent masses.

5. Finally I desire to record my strong impression that the human larynx develops as a unit; that its cartilages and muscles are dependent or interrelated with the pharynx or the tongue in their development, in no further degree, than is the natural association of one part of the body with a contiguous portion.

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NON-MEDULLATED NERVE FIBERS IN THE SPINAL NERVES

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SEVEN FIGURES

It is generally admitted that in the cerebro-spinal nerves there are a few non-medullated fibers, supposedly derived from the sympathetic system: but a study of the spinal nerves by various silver-impregnation methods has led me to believe that the non-medullated fibers are very numerous, even exceeding in number those which are medullated. A preliminary note on this subject was published in May, 1909, and some of the preparations were demonstrated at the meeting of the American Association of Anatomists (December 28, 1909). Similar observations have been made on the nervus intermedius by Weigner ('05).

MATERIAL AND METHODS

Observations have been made upon the spinal nerves of men, dogs, cats, rabbits and rats. In animals the sciatic and the lower cervical and lumbar nerves including their roots and dorsal root ganglia were examined. Two human sciatic nerves which had been obtained fresh from amputated limbs were also studied. For these I am indebted to Dr. G. D. Scott and Dr. Wm. Speidell.

For the demonstration of the myelin sheaths the Pal-Weigert method and the method of Stroebe were used. These are so well known that no account of the technique need be given. It should be said, however, that in the differentiation of the Pal-Weigert preparations great care was exercised not to decolorize any of the medullated nerve fibers. Osmic acid could not be used as a stain for the myelin sheaths because it will not penetrate to the center of a nerve large enough to give a satisfactory preparation by any of the silver methods.

For the demonstration of the axons those methods which cause a deposit of reduced silver in the axons give by far the best results. Of these procedures three were successfully applied, Carjal's method, Bielschowsky's method, and a new method, developed during the course of this investigation, which involves the use of pyridine. All three are closely related and give similar pictures. Cajal's method, which is the original and of which the others are only modifications, is carried out as follows. Pieces of fresh nerve (preferably a large nerve like the human sciatic) are placed for two days in absolute alcohol containing 1 per cent of concentrated ammonia; washed one to three minutes in distilled water; placed for three to five days in a 1½ per cent aqueous solution of silver nitrate in the dark at 37° C; washed three to five minutes in distilled water; placed for one to two days in a 1 per cent solution of hydroquinone in 10 per cent formalin. The tissue is then imbedded in paraffin and cut into sections, which, after mounting, are ready for examination. The preparations may sometimes be improved by treating the sections on the slide with a neutral gold bath containing five drops of a 1 per cent gold chloride solution to each 10 cc. of water. This method is uncertain in its results and only occasionally gives satisfactory preparations of the spinal nerves. At best it is only a limited part of the section which is fit for examination. But, when a satisfactory preparation is obtained, the work is well repaid by the clearness with which all the axons are demonstrated.

Bielschowsky's method, for some unknown reason, could not be successfully applied to animal nerves. Very beautiful preparations were obtained by its use on the human sciatic, and these confirmed in every way the findings by Cajal's method. Since it does not seem to be generally applicable the details of the procedure will not be given. They can be obtained from Bielschowsky's own account of his method ('05).

Both of these silver methods are very unreliable in so far as their application to the spinal nerves is concerned. By treating the tissue with pyridine before it is put into silver nitrate the staining of the non-medullated fibers is rendered much more intense. The reaction appears to be very constant and few failures need be anticipated if pure chemicals are used. The method is applicable to much smaller nerves than can be successfully treated with Cajal's method, and gives a more uniform impregnation throughout the nerve. The technique is as follows: The nerve or ganglion is placed in 100 per cent alcohol with 1 per cent ammonia for forty eight hours. (95 per cent alcohol with 5 per cent ammonia will give much the same results but seems more likely to bring out the nurilemma nuclei.) The pieces are then washed for from one-half to three minutes (according to their size) in distilled water and transferred to pyridine for twenty-four hours, after which they are washed in many changes of distilled water for twenty-four hours. They are then placed in the dark for three days in a 2 per cent aqueous solution of silver nitrate at 35° C, then rinsed in distilled water and placed for one to two days in a 4 per cent solution of pyrogalic acid in 5 per cent formalin. Sections are made in paraffine and after mounting are ready for examination.

All of the illustrations shown in this paper were made from Cajal preparations before the pyridine-silver method was developed. The preparations obtained by the latter however do not differ in any essential point from those by Cajal's method which have served as the basis for the illustrations and descriptions in this paper.

HISTOLOGICAL OBSERVATIONS

When a satisfactory Cajal preparation is cut into transverse sections one finds the axons of the medullated fibers stained a light brown (figs. 1 and 7). These are surrounded by a colorless ring, representing the myelin sheath; or there may be a very light yellow stain in the neurokaratin framework. The neurilemma is not clearly differentiated and the axon often appears to have shrunken. Imbedded in the light yellow endoneurium which separates the individual medullated fibers are black dots, which, unless the preparation is very thin, can be seen to be the cut ends of small black fibers. They vary in size. Some are as large as the axons of the smallest medullated fibers, others much smaller. Their grouping is quite characteristic; running for the most part in bundles, they appear on cross section as clusters of dots. Isolated fibers are however not uncommon. The non-medullated fibers are not uniformly distributed throughout the nerve, in some fields only a few scattered groups can be seen; while in others they greatly outnumber the medullated axons.

Oblique sections give the most convincing demonstration of these fibers. In a good Cajal preparation the background is so faintly stained that sections 20μ thick are not at all obscure. In such a preparation it is possible, beginning with the lower surface of the section in focus, to see the sharply defined ends of the cut fibers and by raising the focus gradually follow these same fibers obliquely upward through the section in a somewhat undulating course between the medullated fibers, and on reaching the upper surface of the section see them terminate again in sharply defined rounded dots. This oblique course has been very difficult to reproduce in a drawing but an attempt has been made to illustrate it in fig. 2. In longitudinal sections these fibers can be followed for a considerable distance. They have not been seen to branch, but no special search has been made for branching fibers.

About some of these axons a faint halo can be seen, a ring so delicate that it can be recognized only with the highest magnification. Its significance has not been determined, but two possible explanations must be considered. The rings may be produced by the shrinking of the axon or there may be a definite sheath (aside from the neurilemma) surrounding non-medullated fibers. This view has been upheld by Shiefferdecker and Tuckett. According to the latter these sheaths are destitute of myelin, while according to the former they contain a faint trace of that substance. This opens up an interesting question concerning the structure of non-medullated fibers which will require careful investigation. At the present time we are concerned only with showing that the axons under discussion are identical with those designated as non-medullated, and are entirely distinct from, and in addition to, the medullated axons, which alone have heretofore been taken into consideration in the spinal nerves.

Teased preparations of nerves prepared according to Cajal's method are readily made. After the impregnation and reduction are complete the nerves are dehydrated, cleared and teased in creosote. In such preparations one can see the axon of the medullated fibers surrounded by a faintly stained network of neurokeratin representing the myelin sheath; even on the finest of such fibers the nodes of Ranvier can be found. Among these can be seen the non-medullated fibers running in bundles or alone. Often an axon can be seen running from one bundle to another. Closely applied to them in favorable preparations can be seen elongated nuclei with their long axes corresponding to that of the fiber and resembling neurilemma nuclei on the sympathetic fibers (fig. 3). One non-medullated fiber was followed for a considerable distance and showed four such nuclei; but no trace of a node of Ranvier could be found.

In order to compare the fibers under discussion with the sympathetic non-medullated fibers preparations were made by Cajal's method from the semilunar sympathetic ganglion of a dog. Scattered medullated fibers could be seen in these preparations but the great majority of the fibers were non-medullated and differed only in their grouping from those of the spinal nerves herein described. The non-medullated fibers in the sympathetic ganglion are uniformly and solidly massed together into large fascicles, while in the spinal nerves they are grouped into small bundles widely separated by intervening medullated fibers.

All the methods based upon the reduction of silver in the axon (Cajal's, Bielschowsky's and the new pyridine-silver method presented in this paper) often stain connective tissue fibers. Both the elastic and white fibers have been stained in some of the preparations of the spinal nerves. The white fibers are much too delicate to be mistaken for axons; and the elastic fibers are readily recognized by their isolated course and characteristic branching. The size and characteristic grouping of the non-medullated fibers excludes the possibility of their being confused with connective tissue fibers. Moreover it is only in faulty preparations that the connective tissue fibers are stained. In the best preparations the connective tissue does not appear fibrillar but finely granular; and the axons stand out clearly from this light yellow, finely granular background.

If the fibers which have been described cannot be confused with connective tissue, is it possible that they are small medullated fibers? The existence of many very small medullated fibers in the nerves, and the fact that the Cajal method does not give a satisfactory demonstration of the myelin sheaths make this a very pertinent question. In order to test this possibility a comparison has been made between the axons brought out by Cajal's method and the myelin sheaths stained by the Pal-Weigert method or by that of Stroebe. Since osmic acid does not penetrate well into nerves of a size required to give a satisfactory silver stain, it could not be used for this purpose. It is not possible to take two immediately successive sections and stain one for axons and the other for myelin sheaths, since each method requires a special treatment of a block of tissue. However, sections which in their original position in the nerve were not separated by more than a few millimeters can be compared. The photomicrographs (figs. 6 and 7) are from adjacent sections of a human sciatic nerve. Fig. 6 from a cross-section stained by the Pal-Weigert method shows the myelin sheaths as dark rings, and brings out as large a proportion of small medullated fibers as can be found anywhere in the section. Fig. 7, from a Cajal preparation, shows the myelin sheaths as colorless rings and the axons, both medullated and non-medullated, as black dots. It was photographed from a field especially rich in non-medullated fibers. The magnification was the same in both cases. This enables one to compare the number of myelin sheaths in one section with the number of axons in an adjoining section of the same nerve. Since the area represented in Fig VI contains a high proportion of small medullated fibers, and since the fibers are not more widely separated, than in other parts of this section, or in other sections of the human sciatic, it follows that the photograph represents approximately the maximum number of myelin sheaths for an area of that size in the human sciatic nerve. In fig. 7 there are many more axons than could be ringed by that number of myelin sheaths. The same numerical relations are brought out in figs. 4 and 5, which are camera lucida tracings from the sciatic nerve of the dog. Fig. 4 is from a Stroebe preparation and represents the myelin sheaths as solid black rings. Fig. 5 is from a Cajal preparation and represents medullated axons in stipple and non-medullated axons in solid black. It is clear that there are many more axons than there are myelin sheaths.

These non-medullated fibers have been demonstrated in the cervical and lumbar nerves of animals (dogs, cats, and rabbits) close to the ganglia, as well as in the longitudinal fiber bundles within the ganglia, both on the peripheral side when they are about to join the ventral roots and on the central side just before the dorsal root fibers leave the ganglion. These fibers correspond in every way with those seen in the sciatic nerves. From this evidence alone it would seem probable that at least a considerable proportion of these fibers seen in the peripheral nerves either arise in, or pass through the spinal ganglion. Satisfactory preparations of the ventral roots and of the dorsal roots beyond the ganglia have not been obtained. This is probably due to the small size of the radicles of which the roots are composed. This difficulty in technique has greatly retarded the work of tracing these fibers toward the central nervous system.[1]

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[1] Since this paper was written Mr. Chase, working in this laboratory with the pyridin-silver method, has obtained a very beautiful demonstration of the non-medullated fibers in the dorsal root of a spinal nerve of the dog. He has kindly permitted me to mention this observation in advance of the completion of his investigation of the roots of the spinal nerves.

AN INTERPRETATION OF SOME OF THE LITERATURE ON THE SPINAL GANGLION IN THE LIGHT OF THESE OBSERVATIONS WITH REMARKS ON THE SOURCE OF THE NON-MEDULATED FIBERS.

In order to show how the presence of these non-medullated fibers clears up many obscure questions in previous histological, numerical, and experimental observations on the primary sensory spinal neurones it will be desirable to present a summary of some of the known facts concerning the spinal ganglion cells and their processes. At the same time evidence will be presented to show that the non-medullated fibers are the axons of the small cells of the spinal ganglia. The accurate data, relating to the numerical relations in the spinal nerves, which have been accumulated under the direction of Dr. Donaldson, formed the starting point for this investigation; as they now form one of the chief arguments in favor of the correctness of the observations herein recorded.

1. *Axons of the spinal ganglion cells*

The vast majority of the cells of the dorsal root ganglia are associated with axons which branch dichotomously into central fibers, which run in the dorsal root, and peripheral fibers, which run in the nerve. A careful reading of Dogiel's book "Der Bau der Spinalganglien" ('08) will show that our views concerning this fundamental point, while obscured by an astonishing wealth of detail, have not been materially altered. In six (I, II, V, VI, VII, X) of his eleven types this branching occurred. In type IX the cells were bipolar with centrally and peripherally directed fibers. In types III, IV and XI he was unable to determine the destination of the axon, but in only one type (type VIII) did he find the distribution of the axon different from that which has been commonly accepted.

Nissl's ('03) idea that the dorsal root fibers are independent of the spinal ganglion cells, merely passing through the ganglion, has received no support and has already been sufficiently discussed in a previous paper ('08). We will be not far from right if we adhere to the long accepted teaching that the axons of the spinal ganglion cells divide dichotomously into central and peripheral fibers.

2. Axons of the small spinal ganglion cells

In a paper published in 1898 Dogiel showed that the small cells of the spinal ganglion were associated with non-medullated fibers. He says:

> In addition to their size the only distinction between the cells in question (small cells) and the large cells consists in the fact that from each such cell there always arises a single extremely slender process which remains non-medullated throughout its entire course . . . . and finally divides in the forms of a T or Y into two slender varicose threads. I have succeeded . . . . in demonstrating that the chief processes of the small cells and the fibers that arise from their division retain the character of non-medullated fibers so far as they can be traced in the ganglion, the dorsal root and toward the point of union of the ventral and dorsal roots.

In Dogiel's recent book ('08), speaking of the cells of type I, he says that the small cells have non-medullated axons. He also states that the cells of type X are all small and that their axons are all non-medullated. Cajal ('06) working with his silver method, was able to confirm those observations of Dogiel's and to show that the axons of the small cells of the spinal ganglia are non-medullated and divide in the form of a T or Y into central and peripheral fibers. The axons of the large cells are medullated.

3. Observations on the structure and functions of the small cells

This difference between their axons is only one of the many differences between the large and small cells. Special emphasis has been laid on this point in another paper (Ranson '08) and we need only summarize briefly some of the peculiarities of the small cells and mention some of the theories to which these peculiarities have given rise in order to show that the necessity for some explanation of the significance of these little cells has long been felt. It should be remembered that in the opinion of the writer their true significance lies in their being the cells of origin of the non-medullated fibers of the spinal nerves. The small cells differ histologically from the large cells in being small, angular, possessing a relatively small amount of cytoplasm, and in staining more intensely with the diffuse protoplasmic dyes (v. Lenhossek '86 and '95, Fleming '95, Cox '98, Hatai '00). Ranson ('08) has shown that when the spinal ganglion is stained by a modification of Doaggio's 'Method VII' the large and small cells present a very striking contrast; the cytoplasm of the large cells is colorless except for a network of blue threads while the cytoplasm of small cells is a deep violet and almost free from such threads. There are transitional cells of medium size presenting some of the characteristics of both types.

That there is also some physiological distinction between the two types is indicated by the experiments of Hodge ('89). He noted that after electrical stimulation of a nerve it is chiefly the large cells which show the effect of fatigue, the small cells for the most part being unaltered.

Various theories have been advanced to account for the presence of the small cells. Rawitz ('80) considered them young developing ganglion cells, the immediate result of a supposed—but confessedly undemonstrated—cell division. Since mitosis occurs rarely if at all in the spinal ganglia during extra uterine life this theory is not tenable. Bühler ('98) believed that the small cells served as a reserve and that when a large cell degenerated a small one increased in size and took its place. Hatai ('02) has shown that this view cannot be held since the total number of cells remains constant from birth to maturity.

Hatai ('02) has also shown that during the growing period of the animal the small cells are constantly being transformed into large ones. He concludes that they are "in a growing state or in a more or less permanently immature condition." After having made these valuable observations he assumes without adequate evidence that many of these small cells have no axon and are by inference functionless. Hardesty ('05) agrees in considering them 'anaxonic' or "latent cells which have not yet developed processes." Contrary to this assumption we have seen that their axons have been described by Dogiel and Cajal. Furthermore v. Lenhossék ('95) says: "There are no apolar cells in the spinal ganglion;" and after a careful search for them Hodge ('89) came to the same conclusion.

V. Lenhossék ('95) has stated well what seems to be a correct view of these cells: "the smaller cells, even indeed the smallest cells, are not to be regarded as functionless rudimentary structures, but as elements which just as truly as the large cells are functional parts of the nervous mechanism: we find them associated just like the large cells with a process which divides in the typical way," (into a central and a peripheral fiber). To this must be added Hatai's observation that during the growing period they are capable of transformation into large cells.

4. Numerical relations between the large and small cells

Hatai ('02) enumerated separately the large and small cells of the spinal ganglion of the white rat and showed that in the adult animal the ratio between the large and small cells was, in the fourth cervical ganglion as 1 is to 1.4, in the fourth thoracic as 1 is to 1.5 and in the second lumbar as 1 is to 1.5. In these three ganglia of the adult white rat the small cells constitute approximately 60 per cent of the total number. Warrington and Griffith ('04), who worked with the second cervical nerve of the cat, state that the small 'obscure' cells represent 68.1 per cent and the small 'clear' cells 1.9 per cent or a total of 70 per cent. In both investigations the structure as well as the size was taken into consideration in determining which were large and which were small. From these observations we may safely say that in the cat and rat nerves studied about two-thirds of the spinal ganglion cells may be classified as small.

5. Numerical relations between the spinal ganglion cells and afferent fibers

It has been shown by Hodge ('89), Bühler ('98), Hatai ('02), Hardesty ('05), and Ranson ('08) that the spinal ganglion cells are much more numerous than the medullated afferent fibers of the associated dorsal root. Since the "distal excess" is small the number in the dorsal root would correspond closely to the number of medullated afferent fibers in the nerve just beyond the ganglion.

The ratio of cells to fibers varies with the animal and the nerve stiuded, but in the second cervical nerve of the white rat Ranson ('08) found a very constant ratio of 3.2 cells for each medullated afferent fiber in the dorsal root. This is explained by the fact that the large cells which as already stated represent about one-third of the total number are associated with medullated fibers, while the small cells (two-thirds of the total number) are associated with non-medullated fibers.

We wish now to present evidence to show that the axons of the small cells are represented by the non-medullated fibers which we have demonstrated in the peripheral nerves.

6. *Axonal reaction in the small cells of the spinal ganglion following lesion of the associated nerve*

All who have investigated the subject, Fleming ('97), Cox ('98), Köster ('03), Lugaro ('04), Warrington and Griffith ('04), and Ranson ('09) agree that the vast majority (anywhere from 85 per cent to 100 per cent) of the cells in the spinal ganglion show chromatolysis after division of the associated nerve. Köster ('03) has maintained that since nearly all the cells react as if their axon had been cut, the numerical results showing many more cells than nerve fibers must be incorrect. But the counts of the medullated fibers and ganglion cells have been too often confirmed to be open to doubt; and we must explain the discrepancy between the numerical and experimental results, not by denying the correctness of either, but by the fact that the non-medullated axons of the small cells were not taken into consideration in the enumeration.

Lugaro ('04), Cox ('98), and Ranson ('09) have shown that the small cells show typical axonal reaction and react earlier and more energetically than the large cells. Ranson ('09) has also demonstrated that in the rat few of the large cells show irreparable chromatolysis while the small cells degenerate and disappear in large numbers. "It is in these small cells that the most extreme alterations are to be found. The nucleus is strikingly eccentric; in most cases it causes a distinct bulging of the cell outlines, and in many it appears to be indenting the cell from without. The chromatic substance is completely dissolved except for a dense ring which persists at the periphery of the cell and a small clump sometimes found near the nucleus. Even at this stage, five days after the operation, it is clear that some of these small dark cells have disintegrated." A differential count made twenty days after the operation showed the same number of large cells in the normal and 'operated ganglia' (an average of 29 to a section) but the number of small cells was reduced by one-half (from 42 to 19.5 per section). From these observations it is clear that following the division of a nerve the small cells of the associated ganglion react exactly as if their axons ran into the nerve and had been divided when the nerve was cut.

Confirmation of these results is to be had from another series of experiments (Ranson '06). The dorsal ramus of the second cervical nerve was cut in eleven white rats, eight of which were 12 days old and three were adult specimens 140 days old. Enumerations were made of the spinal ganglion cells in nine and of the dorsal root fibers in ten of these animals after from two to four months. Similar counts were made on normal animals for control. There was a reduction in the number of spinal ganglion cells as a result of the operation from an average of 8451 in four normal specimens to 4124 in nine 'operated' ganglia. This loss was a very constant one and there was no greater variation among the operated ganglia than among the normal ones. It was shown that in the operation on 12 day old rats approximately 1500 medullated afferent fibers were cut and in the operation on rats 140 days old approximately 2500. This variation in the number of medullated axons cut had no influence on the number of cells degenerating, and moreover, it is obvious that even the larger number is not sufficient to explain the degeneration of over 4,000 ganglion cells. More surprising still was the observation that after more than 4000 ganglion cells had completely degenerated there was an average loss of only 473 dorsal root fibers. Since the dorsal root fibers have their cells of origin in the spinal ganglion this last statement seems incredible.

These observations are, however, easily explained on the basis of the facts already presented. The excess of the number of cells destroyed over the number of medullated axons cut in the division of the nerve is explained by the presence of the non-medullated axons. And the fact that the majority of the medullated fibers of the dorsal root remained intact after the loss of more than half of the spinal ganglion cells is explained by the fact that the cells which disappear are chiefly small cells whose axons both in their central and peripheral prolongations are non-medullated

In concluson it may be said that there are in the spinal nerves a very large number of non-medullated nerve fibers. They are in fact more numerous than those which are medullated. While a few are no doubt derived from the sympathetic system by way of the gray rami communicantes, the experimental and numerical evidence just presented makes it clear that at least a great many of them are the peripheral branches of the axons of the small cells of the spinal ganglia. We now have a satisfactory explanation for the excess of spinal ganglion cells over medullated afferent fibers and for the chromatolysis which appears in practically all the cells of a spinal ganglion after its associated nerve has been divided.

It is obvious that this investigation raises more questions than it settles. We have yet to determine the central course of these axons, whether any pass by the ventral root, and what proportion come by way of the rami communicantes. It will also be necessary to determine whether they are distributed chiefly to the skin muscles or blood vessels. If they go chiefly to the skin it may be possible that they are associated with some one or more of the varieties of cutaneous sensation. It will be necessary also to repeat the Wallerian experiment and study the degeneration of these fibers. In the pathology of the peripheral nerves it will be of interest to learn how these fibers behave in tabes dosalis, neuromuscular atrophy and other conditions in which the pathology, so far as it relates to the peripheral nervous system is very obscure.

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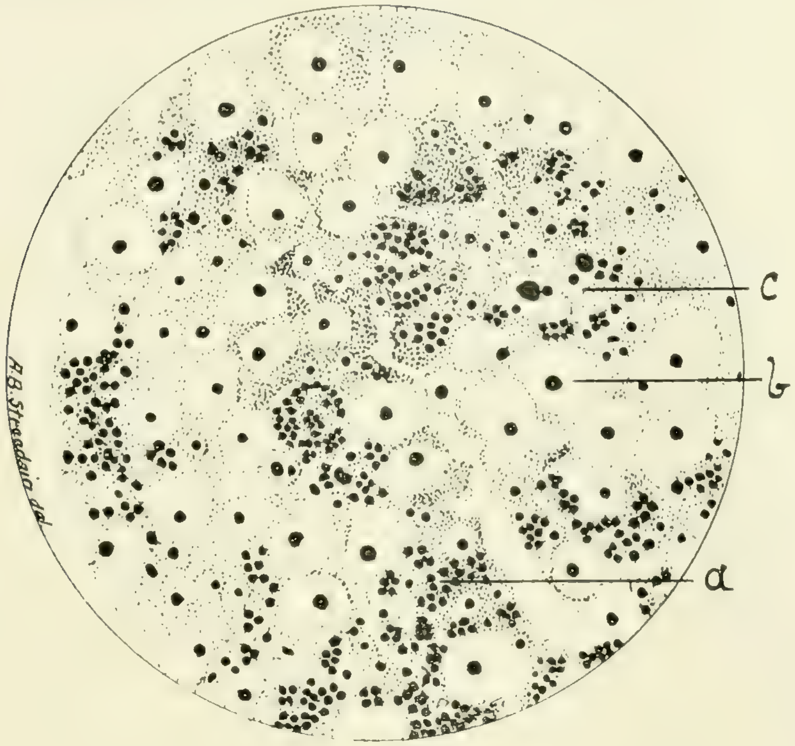
PLATE 1

EXPLANATION OF FIGURES

1 From a transverse section of a human sciatic nerve. Cajal's method. *a*, non-medullated fibers; *b*, large medullated fibres; *c*, small medullated fiber. × 600.

2 From a cross section through a human sciatic nerve. Cajal's method. On one side the fibers are cut transversely (*a*) and on the other obliquely (*b*). Zeiss Compens. Ocu. 4; Obj. 4 mm.



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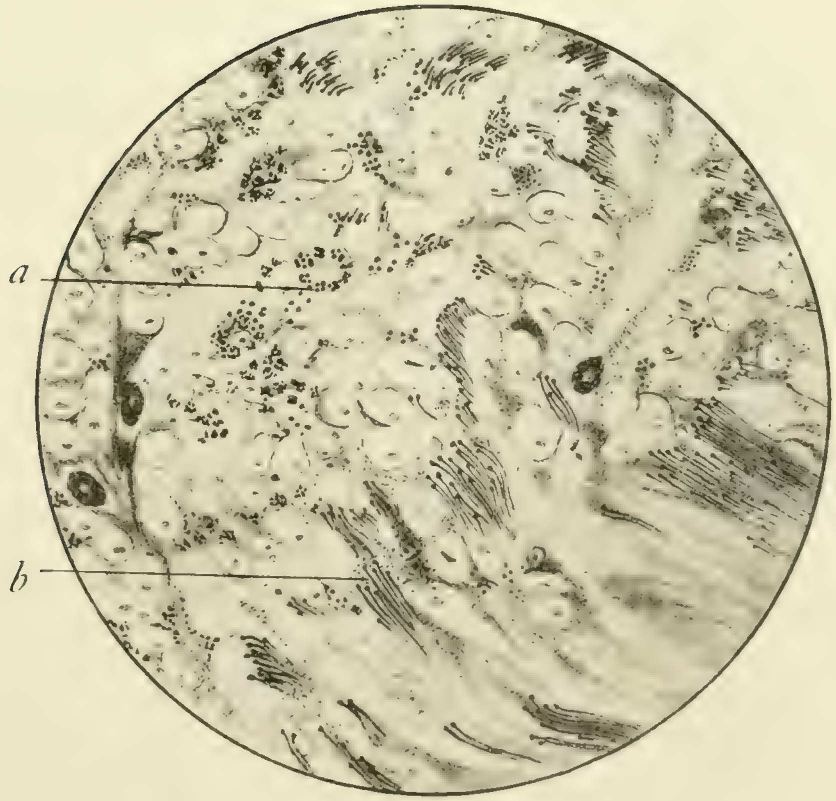

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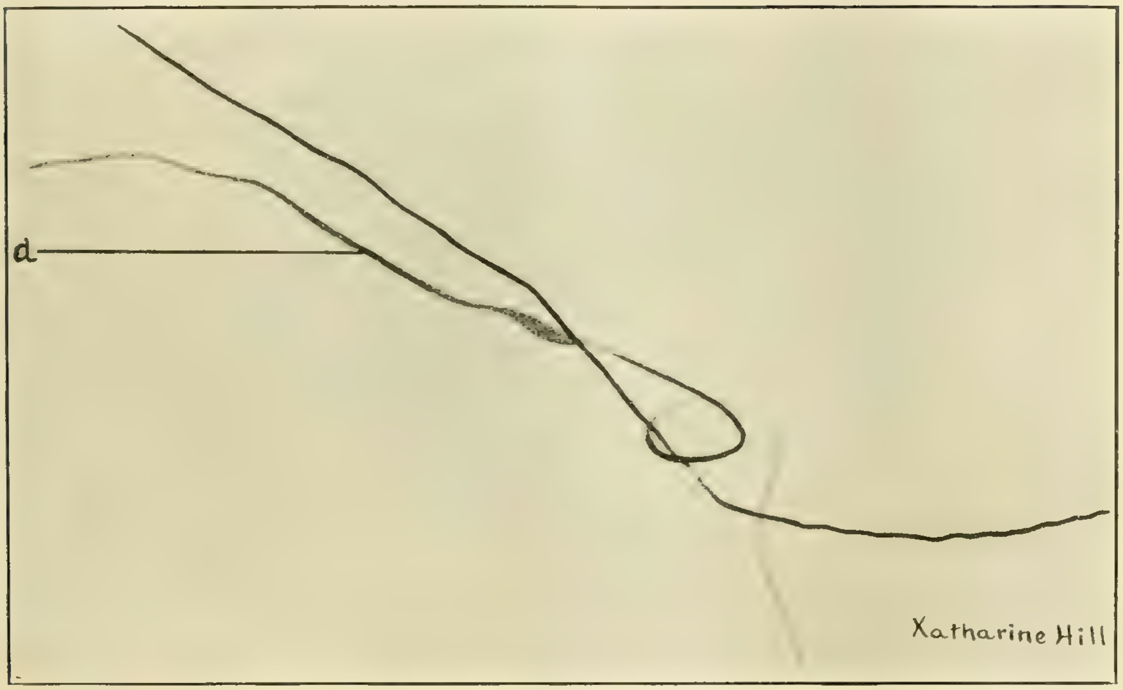
PLATE 2

EXPLANATION OF FIGURES

3 From the sciatic nerve of a dog, Cajal's method, teased. Note the nucleus on the non-medullated fiber (*a*). Zeiss Compens. Ocu. 12; Obj. 4 mm.

4 Camera lucida tracing of a section of the sciatic nerve of a dog. Stroeebe's method. *b*, large medullated fiber; *c*, small medullated fiber. Spencer Ocu. 8 ×, Obj. 2 mm.

5 Camera lucida tracing of a Cajal preparation of the sciatic nerve of a dog. *a*, non-medullated fibers; *b*, large medullated fiber; *c*, small medullated fiber. Spencer Ocu. 8 ×, Obj. 2 mm.



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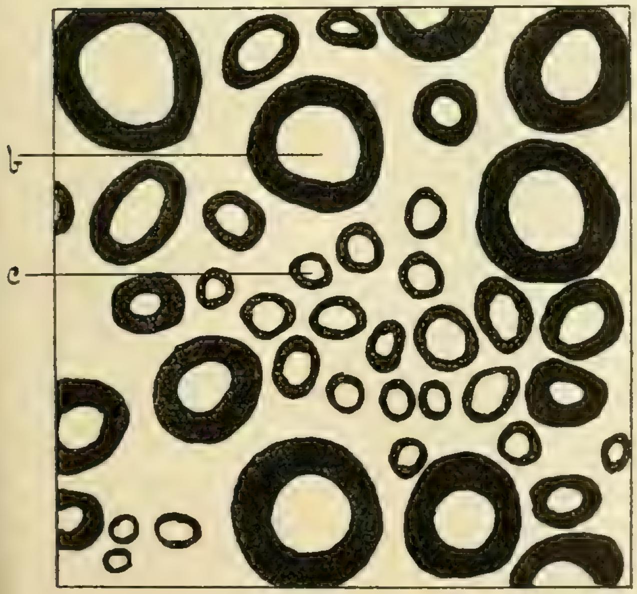

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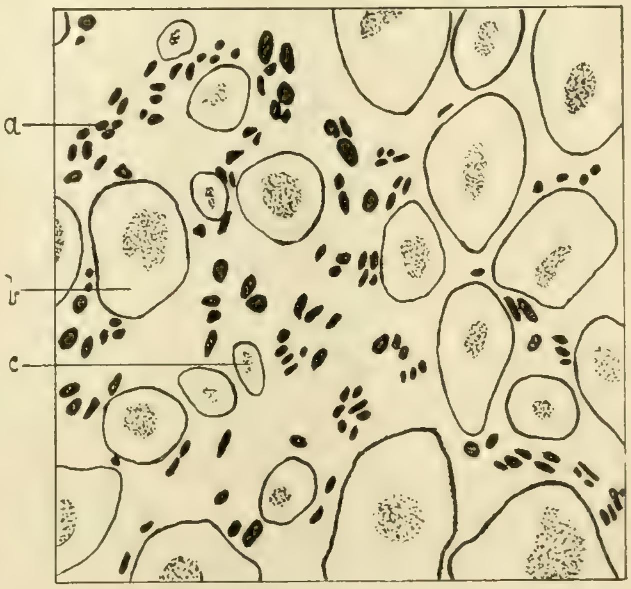

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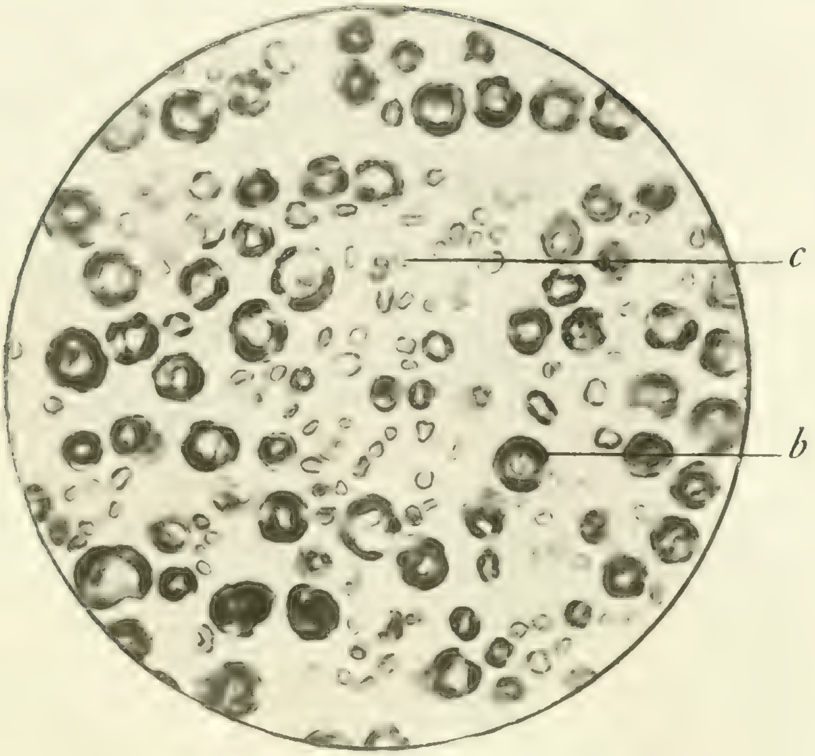
PLATE 3

EXPLANATION OF FIGURES

6 Photomicrograph from a cross section of a human sciatic nerve. Pal-Weigert method. *b*, large medullated fibers; *c*, small medullated fibers. × 600.

7 Photomicrograph from a cross section of a human sciatic nerve. Cajal's method. *a*, non-medulated fibers; *b*, large medullated fiber; *c*, small medullated fiber. × 600.



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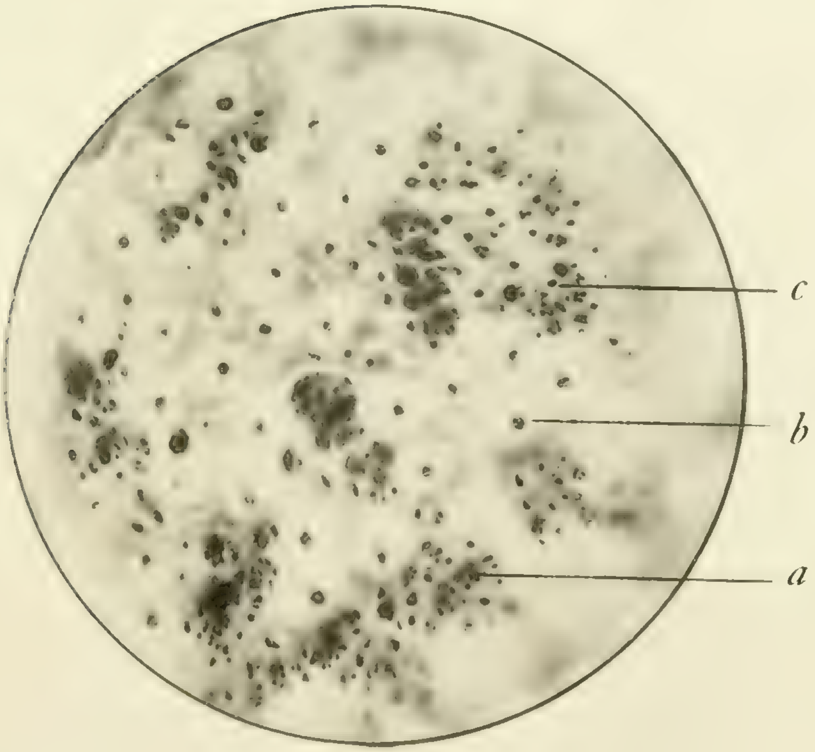

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THE EARLY DEVELOPMENT OF THE MAMMALIAN STERNUM

R. H. WHITEHEAD AND J. A. WADDELL

From the Anatomical Laboratory of the University of Virginia

EIGHT FIGURES

In the text-books of human anatomy it is usually stated that the sternum is formed by the union in the median plane of two longitudinal cartilaginous bars, which are derived on each side from a fusion of the ventral extremities of the embryonic ribs; and that, after this union is established, the costal cartilages are segmented off from the sternum with the formation of costo-sternal articulations. This account is based chiefly upon the studies of Ruge[1] in 1880, although these had to do almost entirely with the sternum after chondrification had begun, that is to say, with a comparatively late stage of development.

The accuracy of this account, with respect to some of its details at least, was first questioned by Paterson.[2] This author describes the sternal anlage in a human embryo 'in the second month' as consisting of an aggregation of mesoblastic cells in the median line of the anterior (cranial) part of the thoracic wall. There is no indication, he says, of bilaterality in the mass. The ventral ends of the clavicles and ribs are composed of cartilage. The first three ribs 'join' the cellular sternum, the fourth and fifth join those above them, while the sixth and seventh have free pointed ends. The meaning of the word 'join' is not entirely clear, but subsequent use of the term in the article indicates that the author does not intend to imply fusion of these structures.

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[1] Ruge, G. 1880. Untersuchungen ueber Entwicklungsvorgaenge am Brustbein und an der sterno-clavicular Verbindungen des Menschen. Morph. Jahrb. Bd. 18.

[2] Paterson, A. M. 1900. The sternum, its early development and ossification in man and mammals. Jour. Anat. and Physiol. vol. 33.

This observation led Paterson to make a study of the development of the sternum in embryos of the rat. In the rat of 9 mm. he finds the sternal anlage as a structure quite similar to that just described in the human embryo—a median mass of cells in the anterior part of the thoracic wall without bilateral subdivision except in front, "where the cells are consolidated into two horns which are concerned with the formation of the clavicles and sterno-clavicular articulations and with the anterior parts of the presternum." The sternal anlage is not connected with that of any of the costal cartilages. In the embryo 10 mm. long the sternum is still cellular. Here there is added to the single median anlage of the presternum the anlage of the mesosternum in the shape of two strands of cells which diverge caudally. The strands are joined by the ventral extremities of the first six ribs, but, he states, there is an obvious difference between the cells composing the two structures. Later on the two cellular strands unite with each other in the median plane forming an unpaired mass of cells which is connected with the first seven ribs on each side. Ultimately the cartilaginous sternum of the rat is laid down as a single median band separated from the clavicles by connective tissue, but in complete fusion with the first seven costal cartilages on each side.

Paterson's observations on the human embryo are probably of little value; but his findings in the rat led him to challenge the traditional account of the development of the sternum. In a subsequent article[3] he holds that the anlage of the sternum is at first single, median in position, and directly continuous with the mass of cells which is to form the shoulder-girdle on each side; and that the shoulder-girdle and the presternum are derived from the same elements. This anlage of the presternum bifurcates into two strands, which grow caudalward and become connected secondarily with the ventral extremities of the ribs. Thus in the final analysis, according to Paterson's view the sternum is derived from the shoulder-girdle.

[3] Paterson, A. M. 1902. The development of the sternum and shoulder girdle in mammals. Brit. Med. Jour., vol. 2, p. 777.

Kravetz[4] has studied the development of the sternum in the pig, using embryos which measured from 24 to 50 mm. in length. In the 24 mm. embryo he describes the sternal anlage as consisting of two bands of condensed mesenchymal cells which are fused with each other at their anterior extremities, while caudally they diverge and extend as far as the level of the ventral extremity of the seventh rib on each side. He denies that there is any true continuity of tissue between the ventral extremities of the ribs, which are in the early stage of chondrification, and the sternal anlage; indeed, the first rib, he states, does not reach the sternal anlage at all. Later on the sternal bands become differentiated into the cartilaginous sternal bars, chondrification proceeding faster in their more cranial portions; and only after this differentiation "tritt eine engere Verbindung der Leisten mit der Ventralenden der Rippen ein." This stage, he thinks, was incorrectly regarded by Ruge as primary.

Finally, Charlotte Mueller[5] in the course of an extensive study of the development of the thoracic walls in man has made very careful observations upon the development of the sternum. In an embryo of 13 mm. she could find no evidence of the sternum, but in one 17 mm. long the anlage was present in the form of two widely separated bands of precartilaginous tissue—the term 'precartilaginous' being used in the chronological and not the histological sense. These bands were connected with each other at their cranial ends by a bridge of less closely aggregated mesenchymal cells. Caudally they diverged and were in direct continuity with the ventral extremities of the first seven ribs, the tissue of the ribs shading off gradually into that of the sternal anlage without any demarcation. In an embryo 15 mm. long she found much the same condition as in the preceding case, except that the bands were not so widely separated, their cranial ends being almost in contact with each other; so that this embryo presented a more advanced stage of sternal formation than the preceding one. In an embryo of 23 mm. the sternal bands had fused with each other at their cranial ends in the region of the first pair of ribs. The anlage of the ensiform process was seen here for the first time as a caudal prologation of the sternal band on each side. Histologically the bands now showed chondrification from the level of the first to the fifth rib inclusive, and there was distinct continuity of tissue between the ends of the ribs and the bands, cartilaginous in the more cranial portion, precartilaginous in the caudal part. Her study of the later stages confirms Ruge's account, and need not be reviewed here. From her observations Mueller concludes that the sternal bands originate directly from the ventral ends of the first seven ribs; and she homologizes the bridge of mesenchymal cells which connects the cranial extremities of the bands in the early stages with the episternum of other forms.

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[4] Kravetz, L. P. 1905. Entwicklungsgeschichte des Sternums und des Episternalapparates der Sauegethiere. Bull. de la Soc. Impér. des Natural. de Moscou. Année 1905, nos. 1–2.

[5] Mueller, Charlotte. 1906. Zur Entwicklung des menschlichen Brustkorbes. Morph. Jahrb. Bd. 35.

From this review of the literature it is seen that the earliest stages of the sternum as observed by Paterson, Kravetz, and Mueller, while not identical, were still very similar; and yet these authors give very different interpretations of their observations. The median portion of the anlage, which Mueller regards as the homologue of the episternum, Paterson considers to be the earliest part of the entire anlage, and thinks that the two sternal bands grow caudalward from it; this median portion he thinks is derived in its turn from the shoulder-girdle. Kravetz, on the other hand, apparently attaches no special morphological significance to it in the pig. Again, Mueller believes that the sternal bands are derived directly from the ventral ends of the ribs, while Kravetz and Paterson hold that the connection of the bands with the ribs is purely secondary. It has seemed clear to us that none of these investigators has had the earliest stages of the sternum before him, and that justifiable conclusions could be reached only through a study of such stages. Accordingly we have undertaken to find such stages in several mammals.

MATERIAL EMPLOYED

We have studied first the pig, thinking that the appearances at the cranial end of the anlage would be less complicated in this form owing to the absence of the clavicles; and then have followed this with an examination of corresponding stages in the cat, in which animal the clavicle is a rudimentary bone not articulating with the sternum. Finally we have studied the development of the sternum in human embryos, the clavicle here reaching its full development.

Observations in pig embryos

We shall begin the account of our findings by a description of the sternal anlage as presented by a pig 24 mm. long.[6] The anlage is first encountered about 12 sections anterior (cranial) to the level of the ventral extremities of the first ribs as an aggregation of mesenchymal cells lying transverse to the median plane of the body. At the level of the first ribs (fig. 1) it is somewhat triangular in cross section, with the apex of the triangle directed ventralward. Each lateral angle of the base is connected with the corresponding first rib. The rib is in an early stage of cartilage formation, but its extremity is capped by a zone of sclerogenous tissue which fades off into the sternal anlage without any definite demarcation, that is to say, there is direct continuity of tissue between the anlage of the rib and that of the sternum. In the figure the ventral portion of the triangle is labeled 'median anlage' because, as will appear later, it has a different origin from the more lateral portions. As the series of sections is examined proceeding in the posterior (caudal) direction, indications of a division of the triangular mass can be seen beginning on the dorsal aspect, until, just posterior to the level of the ventral extremities of the first ribs, two somewhat crescentic bands of mesenchymal cells united across the median plane by embryonic connective tissue separate out from the mass (fig. 2). Before the

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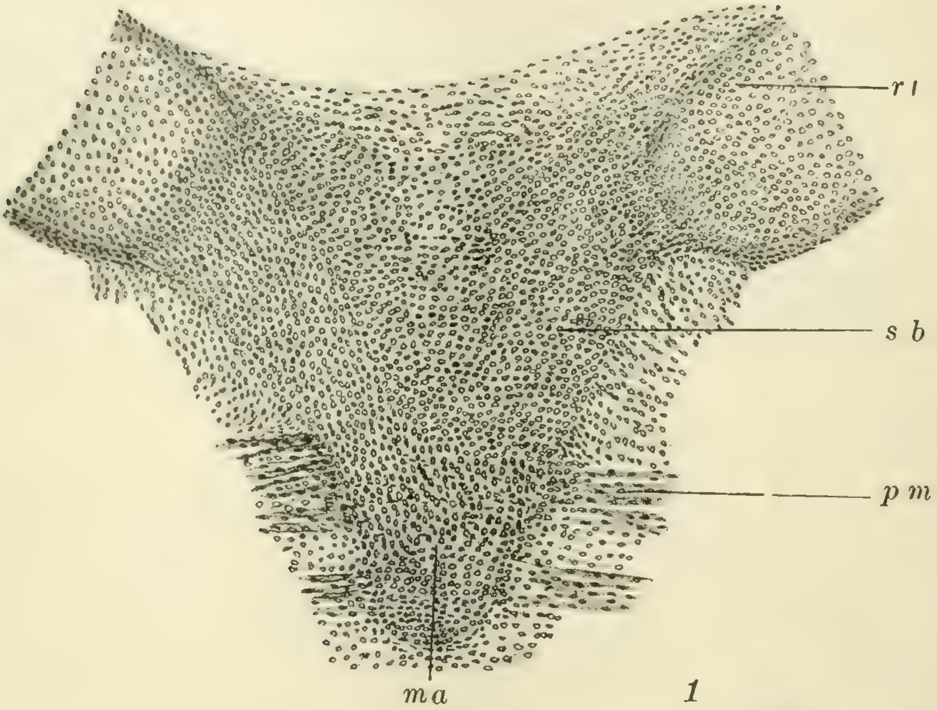
[6] All the embryos were fixed in Zenker's fluid, and the measurements were made in 80 per cent alcohol.



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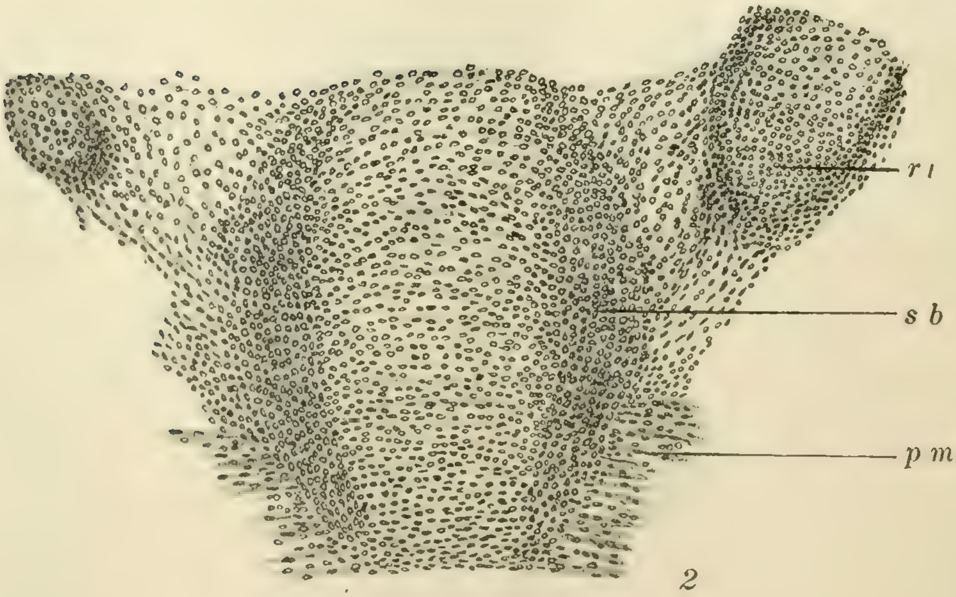

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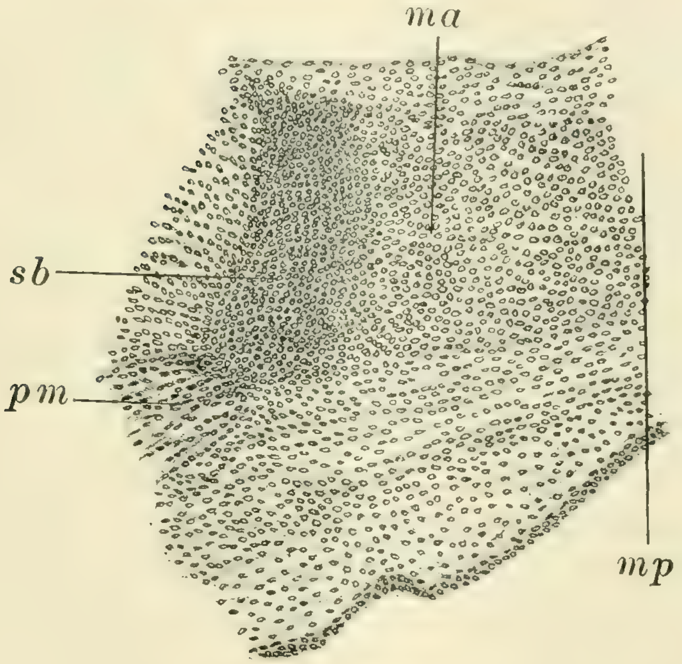
Fig. 1 Pig, 24 mm. Transverse section at level of ventral extremity of first rib; *r 1*, ventral extremity of first rib; *s b*, sternal band; *m a*, median portion of sternal anlage; *p m*, pectoral muscle. × 60.

Fig. 2 Pig, 24 mm. Transverse section caudal to ventral extremity of first rib; *r 1*, first rib; *sb*, sternal band; *pm*, pectoral muscle. × 60.

level of the second rib is reached the band changes shape, the concavity of the crescent being directed lateralward. From here on the anlage consists of two diverging, uninterrupted bands of mesenchymal cells extending, one on each side, as far as the ventral extremity of the seventh rib. The extremities of these six ribs, as in the case of the first rib, are undergoing chondrification, but each is capped by a zone of sclerogenous tissue the cells of which are entirely similar to those composing the sternal bands; and the anlages of the two structures, ribs and sternal bands, shade off into each other without any definite demarcation. It is worthy of note, however, that while the first rib joins the lateral angle of the triangular portion of the anlage (fig. 1), the remaining ribs join in the concavity of the crescent. This embryo thus presents a stage of sternal formation quite similar to the youngest embryo of Kravetz (24 mm.). Kravetz stated, however, that the first rib did not reach the anlage of the sternum in his embryo; and he held, moreover, that the connection between the other six ribs and the sternal band was too feeble to have any special significance. To us, on the other hand, this connection seems marked; and we should not feel able from an examination of this stage alone to conclude that the sternal bands either are or are not derived from the ventral ends of the ribs.

A considerably younger stage was found in a pig 20 mm. long. Here the pericardial cavity extends into the neck, and the ventral extremities of the ribs are separated by a greater interval than in the preceding embryo. A section anterior to the level of the ribs shows that in this region the sternal anlage consists of three parts: the sternal band on each side, and a wide band of less condensed mesenchyme reaching across the median plane and connecting the two sternal bands with each other (fig. 3). The latter are made up of more densely aggregated cells, and consequently appear darker in the stained sections. The anlage in this stage differs from the appearance presented in the preceding embryo mainly in two respects: 1. The sternal bands exist as separate structures well in advance of the ventral ends of the first ribs. 2. There is a median portion of the anlage consisting of less densely aggregated mesenchymal cells connecting the cranial extremities of the sternal bands anterior to the level of the ribs. In other respects there is no essential difference. The sternal bands still extend as diverging, uninterrupted strands of cells along the ventral extremities of the first seven ribs, and are in direct continuity with the tips of these ribs. The condition of the anlage in this embryo seems to accord very well with Mueller's description of the youngest stage found by her in the human embryo. So that in the two embryos just described we have encountered stages practically identical with the youngest stages described by those who have studied the subject before us. But, as we have seen, these stages are susceptible of very different interpretations, and accordingly we have sought for still younger stages.

Such a stage was found in an embryo 22 mm. long. In this pig the sternal bands are still quite distinct anterior to the level of the first ribs but the median portion of the anlage is less well developed. There is an appreciable thickening of the mesenchyme in the region mentioned, but it is not so well defined as in the preceding embryo. The pericardial cavity reaches into the neck and separates the first rib and sternal bands of the two sides. As one proceeds caudalward through the series he is struck by the fact that the ventral extremity of the first rib falls short of the sternal band, and is connected with it only by embryonic connective tissue. Fig. 7 shows the corresponding stage in the cat. The ventral extremity of the second rib approaches the band more closely, and the evidence as to continuity is not so clear as in the case of the first rib; but, if there be any continuity of tissue between the ventral extremity of this rib and the sternal band, such continuity is very slight. The remaining ribs, however, third to seventh inclusive, present the same continuity of tissue between their tips and the sternal bands as was noted in preceding stages. In the more cranial portion of the band, the longest diameter in the cross sections is dorso-ventral in direction, whereas posterior to the level of the second rib this dorso-ventral diameter is much reduced, and the bulk of the anlage tends to lie ventrolateralward from the tips of the ribs; moreover, the band becomes considerably reduced in size in its posterior portion.



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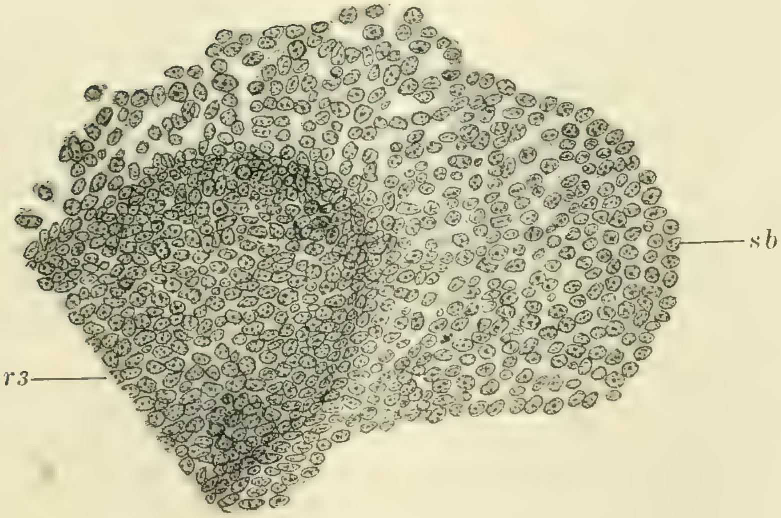

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Fig. 3 Pig, 20 mm. Transverse section 10 sections anterior to level of 1st rib, right side; *mp*, median plane of body; *sb*, sternal band; *ma*, median portion of anlage; *pm*, pectoral muscle. × 60.

Fig. 4 Pig, 18 mm. long. Transverse section at level of 3d rib, right side; *r3*, ventral extremity of 3d rib; *sb*, sternal band. × 200.

We find the next stage in an embryo 18 mm. long. Here the pericardial cavity and the heart are well forward in the neck, and the anterior extremities of the sternal bands are widely separated thereby. There is no evidence whatever of the median portion of the anlage encountered in the previous embryos, so that the sternal anlage now consists of the two lateral portions, or sternal bands, alone. These can be traced from a point about 10 sections of 15 microns each anterior to the level of the ventral extremities of the first ribs to the level of the seventh ribs. Each band, while it presents about the same structure as in the preceding embryos, is smaller, the aggregation of the cells composing it is less dense, and the band is not so well defined from the surrounding mesenchyme. This is particularly true as to the more posterior portion. Still the thickening is quite perceptible, and is uninterrupted throughout. In cross sections the band occupies a position dorsal to the junction of the axilla with the lateral wall of the body, between the pectoral muscle laterally and the pericardial cavity medially. Into this region the ventral ends of the ribs project. They are now entirely free from cartilage, and the cells composing them are very densely packed together, so that the tips of the ribs are very deeply stained in the sections. The first and second ribs fail to reach the sternal band, but the succeeding five are fused with it, and offer the same evidences of continuity with it shown in preceding stages (fig. 4).

In embryos 16 and 15 mm. long the bands were still present, but less clearly defined from the surrounding tissues. In the 16 mm. pig the bands stopped caudally at the level of the fourth ribs; while in the 15 mm. embryo they could be traced no further than the third rib. In both cases the first and second ribs failed to reach the band. In embryos of less length than 15 mm. we were not able to detect any sternal anlage with certainty. There could be found a thickening of the mesenchyme in the region occupied by the band in embryos 15 and 16 mm. long, but it was too poorly defined to allow us to differentiate it from surrounding tissues. Judging from the behavior of the ventral extremities of the first and second ribs in somewhat older stages, we think it probable that a stage exists in which no rib is connected directly with the sternal band, but we were unable to detect such a stage.

To recapitulate: We first find the anlage of the sternum as a blastema occupying a region on each side of the body dorsal to the juncture of the axilla with the lateral body wall between the pectoral muscle laterally and the pericardial cavity medially. At first the blastema is found only in the more anterior portion of this region, reaching as a column of cells from a point well anterior to the level of the first rib as far as that of the 3rd rib on each side in embryos 15 mm. long. It extends rapidly in the caudal direction, so that in the embryo 18 mm. long it reaches as far as the level of the ventral end of the seventh rib. It is uninterrupted throughout its entire extent. Into the region occupied by these sternal bands the ventral ends of the growing ribs project, and soon fuse with the sternal anlage. At first they are not cartilaginous, but are composed entirely of sclerogenous tissue in embryos 18 mm. long. In the earliest stages the first and second ribs do not reach the sternal anlage; it could not be determined whether or not this is also true of the more posterior ribs of the series. However, at the time the embryo is 20 mm. long the ventral ends of the first seven ribs are firmly fused with the sternal band on each side. At first, owing to the projection of the heart into the neck, the two sternal bands are widely separated throughout their entire extent; but as the heart sinks into the thoracic cavity, the bands are allowed to approach each other, the approximation being greatest in the neck, until their anterior extremities finally meet and fuse. Coincident with the sinking of the heart, there appears a third division of the sternal anlage in the shape of a transverse bridge of mesenchymal cells in the ventral wall of the neck which unites the anterior extremities of the sternal bands. As the latter approach each other, this median anlage becomes incorporated with them, and thus assists in the formation of that part of the sternum which lies anterior to the level of the first pair of ribs. At no stage could we detect any evidence of continuity between the sternal anlage and any element of the shoulder girdle.

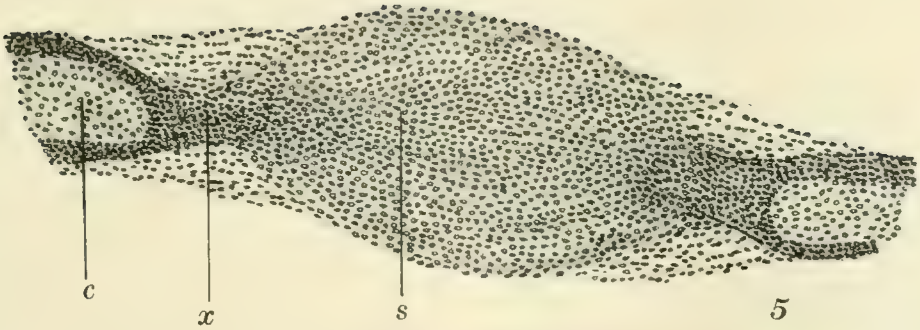
Observations in cat embryos

Our study of the development of the sternum in the cat was made possible by the courtesy of Professor C. F. W. McClure, who gave us the opportunity of examining the beautiful series of cat embryos which form a part of the Princeton Embryological Collection. As the early stages of the sternum in this form agree very well with those already described in the pig, we may make our account brief.

In the cat embryo 18.5 mm. long (series 21) the anterior extremity of the sternal anlage appears as an unpaired, median mass of mesenchymal cells, the cells being arranged more densely on the sides than in the center of the anlage. Just anterior to the level of the ventral extremities of the first ribs the two sternal bands separate out from this median mass, and can be traced as far as the level of the ninth ribs. The ventral ends of the ribs are cartilaginous, but the cartilage is surrounded by a zone of sclerogenous tissue which fuses with the similar tissue composing the sternal bands. Anteriorly these bands lie side by side diverging very slightly until the level of the fourth ribs is reached; but posterior to this region they are separated by the pericardial cavity, and their divergence is more marked. In this posterior region also the bands rapidly diminish in size; and, instead of being crescentic in outline on cross section, they are flattened out so that the long diameter of their sections nearly corresponds in direction with that of the ribs. The clavicles lie almost entirely anterior to the anterior end of the sternal anlage. They contain cartilage, but their medial ends are capped by sclerogenous tissue which is prolonged medialward and caudalward so as to fuse with the sides of the anterior extremity of the sternal anlage (fig. 5). This connection is, however, very slight, extending only through two or three sections (fig. 6). In the progress of development this connection is soon lost. Thus in the embryo 25 mm. long (series 22) there is no connection between the anlage of the clavicle and that of the sternum, and the clavicles lie entirely anterior to the level of the sternum.

The embryo 17 mm. long (series 36) presents the same appearances as those just described, except that the sternal bands are more divergent.

In the embryo 16 mm. long (series 64) the clavicles are still connected to the anterior extremity of the sternal anlage in the way previously noted. The sternum extends about twenty sec-



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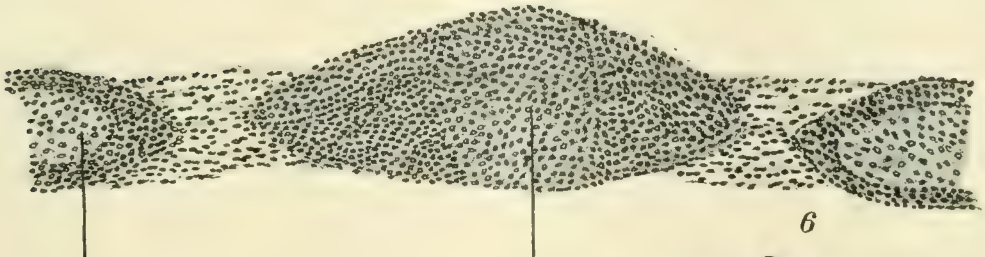

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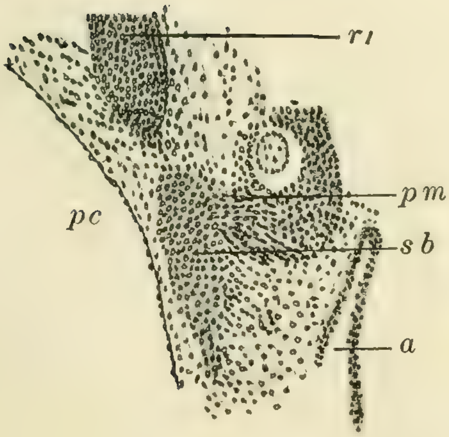

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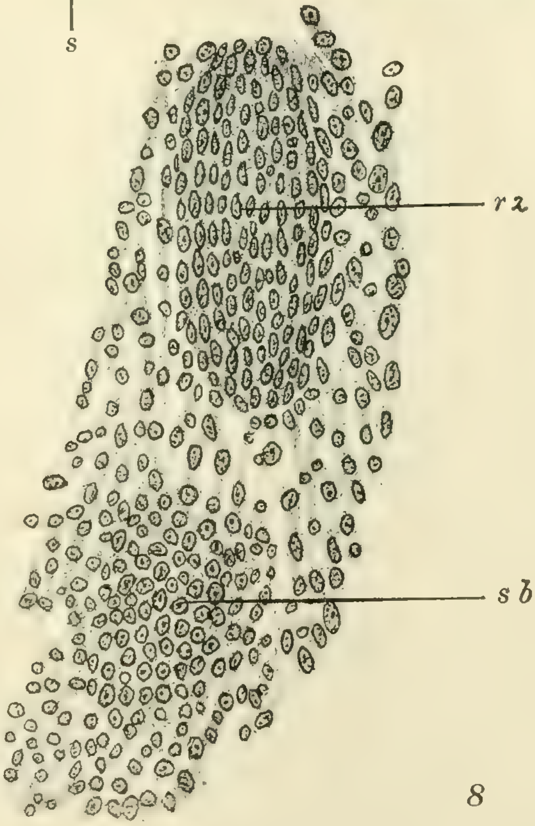

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Fig. 5 Cat embryo, 18.5 mm. long. Cross section of junction of clavicles with sternum. Princeton Embryological Collection, series 21, slide 13, section 2; *c*, clavicle; *s*, sternum; *x*, connection between clavicle and sternum. × 60.

Fig. 6 Same embryo as in fig. 12. Slide 13, section 5. × 60.

Fig. 7 Cat embryo, 12 mm. long. Princeton Embryological Collection, series 401, slide 10, section 24; *r1*, tip of first rib: *sb*, sternal band; *pm*, pectoral muscle; *pc*, peri-cardial cavity; *a*, axilla. × 60.

Fig. 8 Transverse section of sternal band and tip of second rib. Embryo no. 109, 10.5 mm. long, Mall collection. Slide 18, section 1, right side; *r2*, second rib; *sb*, sternal band. × 250.

tions anterior to the level of the first ribs as an irregularly oval mass of cells less densely arranged in the center. The sternal bands separate out just before the first ribs are reached, and extend caudalward as far as the eighth pair of ribs. At first they are circular on cross section, but soon become crescentic, and finally are flattened out so as almost to meet each other across the median plane. They are uninterrupted throughout. Under the low power there is an appearance suggestive of a line of demarcation between the sternal bands and the tips of the first and second ribs, but under the high power the two structures—ribs and sternum—appear intimately united.

In the embryo 14 mm. (series 37) and 13 mm. long (series 35) the median portion of the sternal anlage noted in the pig makes its appearance as a distinct structure just posterior to the level of the clavicles. It consists of a band of cells somewhat larger and less compactly arranged than the cells of the sternal bands stretching across the median plane. Its lateral extremities are fused with the anterior ends of the sternal bands about fifteen sections anterior to the level of the first ribs. The sternal bands are widely separated throughout their extent by the pericardial cavity, and diverge markedly. They occupy a position in the sections dorsal to the line of junction of the axilla with the body wall, and extend as far as the seventh ribs. They are united with the ends of these ribs.

In the embryo 12 mm. long (series 401) the heart and pericardial cavity are well forward in the neck, and there is no evidence of the median portion of the sternal anlage. The clavicles are present as a short bar of cells lying far out in each side of the neck, the two being widely separated by the pericardial cavity. The sternal bands still extend as far caudalward as the seventh ribs. The ventral ends of these ribs are now composed entirely of sclerogenous tissue. The first three fail to reach the sternal bands (fig. 7); it is uncertain if the fourth does, but the remaining three are connected with the bands as in the older embryos.

The embryo 10 mm. in length (series 402) which, however, was from the same litter as the previous one, shows no differences with respect to the sternal anlage, except that the clavicles could not be detected, and the sternal bands could not be traced farther than the level of the sixth pair of ribs.

From this account it appears that the early stages of the development of the sternum in the cat are practically identical with those observed in the pig. There are some minor differences such as the fact that there are nine vertebro-sternal ribs in the cat, and the further fact that there is a rudimentary clavicle in the cat connected for a short time with the anterior end of the sternal anlage. The presence of this clavicle, however, does not influence the median portion of the anlage, which develops in the same way in both forms.

Observations in human embryos

It was desirable to compare the findings in the pig and cat with corresponding stages of a form in which the clavicle attains full development. This we were enabled to do through the kindness of Dr. Mall, who placed the human embryos of the Johns Hopkins Embryological Collection at our service. The embryos which we found adapted to our purpose were those represented by the serial numbers 424, 17.2 mm. long; 296, 17 mm. long; 409, 16 mm. long; 423, 15.2 mm. long; 144, 14 mm. long; 175, 13 mm. long; and 109, 10.5 mm. long. In embryos of less length than no. 109 we were unable positively to identify any sternal anlage.

In the embryo no. 424, 17.2 mm. long, the sternal anlage presents a stage practically identical with the earliest stage described by Mueller. The anterior portion of the anlage consists of a median mass of closely packed mesenchymal cells having the form of a band in transverse sections. On each side this band becomes directly continuous with the sclerogenous tissue forming the medial extremity of the clavicle. Two sections (each 50 microns thick) posterior to this point the anlage is triangular in cross section, the two sides staining more darkly than the central portion and still shading off into the clavicles. Four sections farther the median anlage disappears entirely, and only the lateral portions, or sternal bands, are left. Each of these is in direct continuity with the ventral extremity of the first rib of the corresponding side, and is separated from its fellow by the pericardial cavity. From this point each sternal band extends in the posterior direction as an unbroken cord of cells as far as the level of the seventh pair of ribs. They diverge markedly, and are widely separated by the body cavity. The band tends to assume a position ventro-lateral from the tips of the ribs; the bands and the tips of the ribs are, however, in immediate continuity.

In the remaining embryos of the series which we examined no marked difference in the findings was noted until the embryo 13 mm. long, no. 175, was reached. Here we find a stage very suggestive of that described in the 18 mm. pig and the 13 mm. cat. The pericardial cavity extends far forward, and the sternal bands are consequently still more widely separated at their anterior extremities. The sternal bands could not be traced farther in the posterior direction than the level of the fifth ribs. The ventral tips of the ribs are now non-cartilaginous, and if there be any continuity between them and the sternal bands, it is very slight—too slight in fact to suggest a derivation of the bands from the ribs. Neither the clavicles nor the median sternal anlage could be detected. It is quite possible, however, that the failure to find them was due to the fact that a considerable number of sections had been lost, practically an entire slide full, from the very region in which one would expect to find these structures had they been present in the embryo.

Finally, in the embryo 10.5 mm. long, no. 109, we find the sternal band extending in the posterior direction only as far as the level of the fourth rib. The cells composing the bands are less compactly arranged than in the preceding stages, and the bands are less sharply differentiated from the surrounding tissues; they are, however, uninterrupted throughout. Moreover it is evident that they are not continuous with the tips of the ribs, but are connected with them only by loose mesenchymal tissue (fig. 8).

Thus it appears that in principle the early stages of the development of the sternum are practically identical in the three forms which we have studied; the only difference concerns the relation between the median anlage of the sternum and the blastema of the clavicle. In the case of the pig there is, of course, no question of the relation of the two structures, as the pig has no clavicle; in the cat the sternum is only secondarily and temporarily connected with the clavicle; is there also a stage in the development of the human sternum when the median portion of the sternal anlage is not connected with the clavicle? We think it quite probable that such a stage does exist, though we were unable to find it in the material at our command. However, as already pointed out, this failure may have been due to the loss of a number of sections from the very series in which, *a priori*, we should have expected to find such a stage.

DISCUSSION AND CONCLUSIONS

Our findings make it clear that the theory of Paterson, according to which the mammalian sternum begins as a single median blastema which bifurcates and sends a prolongation caudalward on each side, is untenable. For in all three forms studied by us the appearance of the paired portion of the anlage, the sternal bands, antedates that of the median portion.

The evidence is almost as strong against the view of Ruge and Mueller that the sternal bands are derived from the ventral extremities of the ribs. If the formation of these bands is due to the proliferation of cells composing the ventral ends of the ribs, *i.e.*, if they are segmental in the beginning, one would reasonably expect to find in the early period of their development breaks in their continuity; yet we were not able to detect interruptions in any of the many series of the sternal bands in their precartilaginous stages. A much stronger argument than this, however, is the fact that the more anterior ribs did not reach the sternal bands in the earliest stages that we examined. It is undoubtedly true, in our opinion, that continuity of tissue between the ventral ends of the ribs and the sternal bands does exist at an early stage in the development of the sternum; but in the *earliest* stages in both the pig and the cat such continuity is lacking between the bands and the first and second ribs, while in the human embryo at 10.5 mm. none of the ribs directly reached the sternal band.

As to the derivation of the median portion of the sternal anlage the evidence is not so conclusive. The fact that in both pig and cat embryos this portion of the anlage originates independently of the clavicles enables us to conclude at once that it is not derived from the medial ends of the clavicles. Either of two other interpretations, however, seems possible so far as our observations go: (1) it may be formed *in situ*, or (2) it may be derived from the anterior ends of the two sternal bands by each of them sending a prolongation medialward to join its fellow in the median plane. The fact that we never found this anlage in a paired condition, but always as a single band of cells uniting the anterior ends of the sternal bands leads us to believe that the first interpretation is the more probable.

In conclusion, the conception which we have gained of the early development of the mammalian sternum may be stated as follows: The sternum is developed from three anlagen. First to arise are the lateral portions, the two sternal bands, which make their appearance one on each side of the body wall as a longitudinal blastema dorsal to the junction of the axilla with the lateral wall of the thorax and ventral to the tips of the ribs. The ventral extremities of a certain number of the ribs soon reach this region and fuse with the sternal band. A little later, as the heart begins to sink into the thorax, the third or median anlage makes its appearance as a transverse bridge of cells extending between the anterior extremities of the two lateral anlagen, and the entire blastema now presents the shape of a two-pronged fork. As the heart sinks still more into the thorax, the anterior ends of the lateral anlagen are allowed to approach each other and soon fuse, the median anlage being incorporated with them.

With respect to the morphological significance of the median anlage, we incline to the view that it is the homologue of the episternum of certain of the lower vertebrates, or, perhaps we should rather say, of the prosternum of the monotremes.

PRENATAL GROWTH OF THE PIG

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FIVE FIGURES

Numerous observations on various phases of growth are to be found in the biological literature. Most of those concerning prenatal growth are upon the human embryo, although scattered observations are also recorded on other mammals, and a few on the lower vertebrates. The work presented in this paper was done in the attempt to trace, in the pig, the course of the prenatal growth of the body and especially the relative growth of the various organs. The results are also compared with the course of growth in the human species and in the lower vertebrates, so far as data are available. The work was done in the Anatomical Laboratory of the University of Missouri, under the direction of Dr. C. M. Jackson, to whom I am deeply indebted for his interest, aid and valuable suggestions.

MATERIAL AND METHODS

The material used for this paper consists of 22 litters of pig embryos, comprising about 130 individuals, of which number 105 were used. In most cases, all the pigs of the litter were used, in the others, three or four specimens about the average of the litter were studied. These litters of embryos were secured from the packing houses in Chicago (August 31, 1909), Kansas City (December 27, 1909), and Columbia (at various times, spring 1910). Wherever possible, the litters were worked up in a fresh condition. In the other cases, they were preserved in a 5 per cent aqueous solution of formalin for varying lengths of time (table 1).

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For the ovum, some fifty adult ovaries were examined fresh, by opening the large follicles under a dissecting microscope. The ovum (including zona pellucida) was measured with an eye-piece micrometer, whose divisions had a known value. The largest ovum found is considered nearly, if not quite, the size of the mature ovum. No data were found in the literature as to the size of the mature ovum of the pig.

Of the fetal material secured, the litter at 15 mm. was the smallest which could be conveniently dissected and weighed. The largest litter examined averages 262 mm. in crown-rump length, and is not quite full term. However, the changes between this stage and birth are probably slight, except in the matter of absolute weight. That is, the relative size of the various organs would probably not change much, since the changes are relatively slight during the latter part of the fetal period.

For the data on the adult, a trip was made to the local (Columbia) packing house, and four hogs, probably about ten to twelve months old, were examined and weighed. The individual measurements so secured were averaged, and the averages used in constructing the various curves.

The method used for the fetal and adult material was that of weighing. The crown-rump length was also taken in all cases, as it forms another basis of comparison for the individuals and the different stages.

Each litter is considered as a unit. That is, the individuals in each litter, or the three or four average pigs which were used therefrom, were weighed individually and individual calculations made for the percentage which each organ forms of the whole. Where the intestinal contents were determined (186 mm. and above), their weight was subtracted from the gross body weight, giving the net body weight, which was used in calculating the percentages. The average of the percentages for each organ is then taken for the litter, and this average is used in the table of observations, the minimum and maximum percentages observed in the litter being also indicated in parenthesis. In constructing the various curves of relative growth, it was found convenient to group together certain of the litters closely related in size, the average of the litter averages being taken.

The following measurements were made on each pig: weight and crown-rump length were observed for the whole body; the head, brain, eyeballs, spinal cord, thyroid gland, thymus gland, right lung, left lung, heart, liver, stomach and intestines (with mesentery and contents, also without contents where possible), spleen, pancreas, suprarenal glands, gonads, kidneys and Wolffian bodies, were each weighed separately.

The weights were taken carefully, the organs being placed in a closed glass vessel of known weight. For the larger fetuses, the organs were weighed to 0.001 g. (1 mg.), the body and head being weighed to 0.1 g. For the smaller embryos (18 mm., 25 mm., 37 mm., 41 mm.), the body and head were weighed to 0.001 g., and the organs (except those weighing more than 10 mg. in the 37 mm. and 41 mm. embryos) were weighed to 0.0002 g. (0.2 mg.). For the 15 mm. embryos, the body and organs were weighed to 0.0001 g. (0.1 mg.).

The head was divided from the neck on a plane passing just behind the angle of the mandible and the cranium. Variations in this plane, which to a certain extent are unavoidable, lead to variations in the observed weight, and therefore in the relative size of the head.

The organs were weighed with contained blood, except the heart, which was opened and cleaned of the blood in the cavities. The brain and spinal cord were weighed with the pia mater but without the dura mater.

Since the age of the specimens is unknown, it is impossible to construct accurate curves of growth either for the body as a whole or for the various organs. However, by arranging the figures representing the relative size (per cent of the net body weight) according to the crown-rump length, curves can be drawn which give an approximate idea of the changes in the relative growth of the various organs during prenatal life. But no definite conclusions can be drawn from these curves as to the *rapidity* with which these changes in relative size take place. The only exception to this is in the case of the body as a whole, where some data by Keibel on the age of young pig embryos make it possible to compare the growth in the early part with that in the remainder of the prenatal and with the postnatal period.

A possible source of error lies in the fact that some of the litters were preserved in formalin, while others were studied fresh. It is well known that specimens preserved in formalin show an increase in total weight, amounting sometimes to 10 per cent or 15 per cent of the total. It is, however, improbable that this increase will materially affect the *relative* size of the organs.

OBSERVATIONS

The observations have been condensed into a single table, from which curves expressing the relative growth have been made. A brief discussion of, and explanation for, the table and curves, follows.

Table 1 gives a summary of all the observations on the different litters used. In the first column will be found the serial number of the litter used. In the second column, the manner of preservation. 'Form' indicates a 5 per cent aqueous solution of formalin. The length of time preserved is also indicated. In the third column is given the number of each sex of the individuals used (M–male; F–female).

In the fourth column is given the average crown-rump length, in millimeters, for the litter, the minimum and maximum lengths being given in parenthesis. Similarly in the fifth column is given the average gross body weight for the litter in grams, together with the minimum and maximum. The net body weight is also given for the later stages, in which the intestinal contents could be measured and subtracted.

The sixth column shows the average percentage by weight which the head forms of the entire (net) body weight in each of the various litters. The minimum and maximum percentage found in the litter is given in parenthesis. Similarly in the succeeding columns is given the average percentage of the entire (net) body weight (also minimum and maximum percentage) in each litter for the brain, eyeballs, spinal cord, thyroid gland, thymus, lungs, heart, liver, stomach and intestines with contents, stomach and intestines empty, spleen, pancreas, suprarenal glands, gonads, kidneys and (for the earlier stages) the Wolffian bodies. In the last column is given the average percentage for all the viscera added together, including the brain and spinal cord. Figures for all the viscera were secured by adding up the average percentages for the litter for each of the organs. The ovaries and testes were averaged together, and the resulting figures added. The weight of the stomach and intestinal contents is excluded.

TABLE 1

Observations

LITTER NO.	PRESERVATION	NO. AND SEX OF PIGS USED	CROWN-RUMP LENGTH, AVERAGE (ALSO MIN. AND MAX.)	TOTAL (GROSS) BODY WEIGHT, AVERAGE (ALSO MIN. AND MAX.)
			mm.	*g.*
13	Form-12 mo.	3 (sex?)	15 (15-16)	0.2919 (.2621-.3392)
14	Form-8 mo.	5 (sex?)	18(17-19)	0.701 (.636-.812)
24	Fresh	6 (sex?)	25(25-26)	1.7 (1.57-1.828)
17	Fresh	2M-3F.	37(35-39)	3.25 (2.925-3.47)
21	Fresh	2M.—	42(41-42)	4.97 (4.66-5.17)
8	Form-3 mo.	5M-1F.	58(57-60)	10.33 (9.52-10.88)
2	Form-2 mo.	2M-2F.	82(80-85)	28.2 (26.8-29.2)
5	Form-4 mo.	7M-3F.	86(70-101)	36.54 (22.2-46.4) Average net = 36.38
16	Fresh	1M-3F.	108(105-110)	74.9 (69.6-83.2) Average net = 74.3
23	Form-3-8 mo.	6M-2F.	105(77-115)	90.2 (26.6-116.) Average net = 89.8
4	Form-1 mo.	1M-2F.	108(105-110)	97.0 (82.0-116.5) Average net = 96.2
3	Form-6 wks.	1M-3F.	131(127-135)	116.2 (107.9-124.7) Average net = 115.2
18	Fresh and Form-5 mo.	9M-1F.	125(110-135)	136.7 (96.5-158.7) Average net = 135.1
1	Form-1 mo.	2M-1F.	139(135-141)	153.0 (142-162.6) Average net = 151.1
47	Form-2 mo.	2M-4F.	150(144-152)	216.5 (203.0-239.6) Average net = 214.3
11	Form-1-2 wks.	3M-2F.	156(145-162)	288.8 (265.1-309.6) Average net = 285.4
48	Form-4 mo.	3M-2F.	166(155-176)	334.7 (292.7-377.) Average net = 329.3
10	Fresh	3M-2F.	191(178-202)	395.0 (335.2-442.0) Average net = 388.9
19	Fresh	2M-1F.	215(205-220)	465.0 (348.1-532.5) Average net = 456.2
9	Fresh	2M-3F.	242(224-255)	731.0 (661.-782.5) Average net = 718.2
20	Fresh	—3F.	262(260-265)	745.1 (692.7-825.) Average net = 727.6
Adult	Fresh	2M-2F.	1050	104539. (91021-123614) Average net = 102445.

TABLE 1 (continued)

Average percentage of net body weight (also minimum and maximum)

LITTER NO.	HEAD	BRAIN	EYEBALLS	SPINAL CORD
	per cent	*per cent*	*per cent*	*per cent*
13	26.36(25.64–27.)	8.95(7.57–9.71)	0.14(.11–.19)	
14	29.69(27.46–33.49)	8.97(7.26–10.32)	0.16(.094–.20)	1.87(1.43–2.31)
24	28.95(27.98–29.84)	6.45(4.83–7.58)	0.38(.31–.43)	1.31(.98–1.72)
17	25.44(23.81–27.11)	5.88(5.16–6.71)	0.66(.58–.79)	1.03(.46–1.54)
21	26.61(25.33–27.89)	6.88(6.65–7.10)	0.62(.59–.64)	1.45(1.41–1.48)
8	23.51(22.22–24.72)	6.75(4.39–7.73)	0.95(.82–1.03)	0.86(.78–.99)
2	22.12(21.47–23.61)	4.86(4.71–4.95)	0.87(.82–.89)	0.44(.41–.49)
5	25.33(22.36–30.13)	5.49(3.35–7.33)	1.15(.99–1.22)	0.43(.25–.60)
16	19.65(18.06–21.13)	3.31(2.68–3.95)	0.73(.69–.76)	0.37(.30–.46)
23	21.14(18.83–24.25)	3.55(3.11–4.34)	0.80(.68–1.03)	0.27(.17–.53)
4	22.86(20.95–24.77)	3.98(3.25–4.46)	0.77(.65–.87)	0.24(———)
3	24.74(22.87–25.97)	4.55(4.33–4.87)	0.91(.85–.94)	0.27(.15–.35)
18	22.38(20.28–23.61)	3.60(2.94–4.41)	0.72(.56–.86)	0.23(.13–.42)
1	22.82(22.04–23.96)	4.07(3.60–4.68)	0.78(.74–.81)	0.27(.24–.29)
47	21.17(19.57–23.94)	3.18(2.82–3.48)	0.60(.53–.66)	0.22(.19–.25)
11	23.01(21.56–25.22)	3.55(3.42–3.67)	0.54(.50–.60)	0.21(.17–.27)
48	20.89(19.88–22.61)	3.29(2.98–3.91)	0.45(.42–.48)	0.22(.15–.26)
10	22.67(20.82–25.52)	3.49(3.21–3.82)	0.53(.45–.61)	0.23(.20–.28)
19	21.51(19.68–23.97)	4.08(3.46–5.01)	0.46(.41–.55)	0.26(.20–.32)
9	22.3(21.29–23.31)	3.45(3.25–3.72)	0.34(.31–.39)	0.29(.23–.33)
20	22.29(21.19–23.27)	3.96(3.79–4.09)	0.41(.34–.46)	0.33(.31–.37)
Adult	6.26(4.91–7.41)	0.087(———)	0.011(———)	0.043(.038–.057)

LITTER NO.	THYROID GLAND	THYMUS	LUNGS	HEART
	per cent	*per cent*	*per cent*	*per cent*
13				4.64(4.58–4.74)
14			0.61(?)	3.51(2.73–5.71)
24			0.53(0.42–0.82)	3.26(3.07–3.50)
17			0.99(0.71–1.11)	2.23(2.04–2.39)
21			1.31(1.23–1.39)	2.31(2.16–2.46)
8	0.045(0.032–0.059)	0.099(0.079–0.12)	2.89(2.44–3.13)	2.10(1.62–2.86)
2	0.026(0.018–0.041)	0.054(0.044–0.075)	3.10(2.77–3.46)	1.52(1.46–1.60)
5	0.043(0.032–0.060)	0.13(0.067–0.18)	3.89(3.35–4.62)	1.46(1.24–1.84)
16	0.032(0.027–0.035)	0.15 (0.11–0.19)	2.60(2.22–2.93)	0.83(0.75–0.86)
23	0.027(0.017–0.036)	0.16(0.11–0.20)	2.63(2.29–3.19)	0.88(0.77–1.05)
4	0.031(0.030–0.032)	0.079(0.072–0.086)	3.53(3.35–3.81)	0.88(0.75–1.03)
3	0.032(0.026–0.035)	0.17(0.16–0.18)	3.43(3.17–3.79)	0.88(0.82–0.92)
18	0.038(0.032–0.045)	0.21(0.15–0.30)	2.42(1.76–2.93)	0.66(0.57–0.76)
1	0.031(0.031–0.032)	0.20(0.18–0.23)	3.35(3.10–3.59)	0.89(0.87–0.93)
47	0.023(0.016–0.028)	0.22(0.18–0.28)	2.35(2.01–2.56)	0.72(0.56–0.87)
11	0.024(0.021–0.027)	0.22(0.19–0.24)	2.39(2.26–2.70)	0.72(0.66–0.83)
48	0.016(0.011–0.023)	0.24(0.21–0.28)	2.50(2.05–2.70)	0.48(0.44–0.51)
10	0.021(0.014–0.027)	0.24(0.19–0.28)	2.74(2.62–2.93)	0.67(0.62–0.73)
19	0.035(0.028–0.039)	0.38(0.31–0.51)	3.03(2.79–3.48)	0.99(0.87–1.14)
9	0.024(0.019–0.028)	0.34(0.27–0.47)	2.19(1.80–2.68)	0.83(0.73–0.94)
20	0.026(0.025–0.029)	0.37(0.29–0.52)	2.05(1.83–2.23)	1.03(1.0–1.06)
Adult	0.0039(———)	(?)	0.69(0.42–0.85)	0.32(0.29–0.35)

TABLE 1 (continued)

Average percentage of net body weight (also minimum and maximum)

LITTER NO.	LIVER	STOMACH AND INTESTINES WITH CONTENTS	STOMACH AND INTESTINES EMPTY	SPLEEN
	per cent	*per cent*	*per cent*	*per cent*
13	12.25(11.58–12.86)			
14	11.58(9.23–13.59)	0.27(0.17–0.46)		
24	15.88(12.53–17.66)	1.28(0.96–1.62)		
17	15.27(13.67–16.88)	1.35(0.97–1.78)		
21	14.00(13.32–14.68)	1.62(1.48–1.76)		0.016(0.012–0.019)
8	15.63(14.76–16.86)	2.90(2.41–3.67)		0.039(0.027–0.048)
2	10.51(9.39–11.27)	2.51(2.40–2.66)		0.037(0.031–0.044)
5	9.93(6.81–14.82)	2.95(2.36–3.59)	2.51(2.01–3.05)	0.063(0.038–0.084)
16	8.35(8.04–8.83)	2.75(2.47–2.93)	1.92(1.73–2.06)	0.063(0.054–0.073)
23	5.67(4.84–8.34)	2.34(2.12–2.54)	1.91(1.66–2.25)	0.041(0.026–0.050)
4	5.01(3.88–5.66)	2.83(2.50–3.09)	1.98(1.75–2.16)	0.061(0.050–0.077)
3	4.96(4.44–5.60)	2.96(2.78–3.09)	2.07(1.95–2.16)	0.074(0.067–0.081)
18	6.24(5.58–7.07)	3.46(2.84–4.24)	2.14(1.44–2.59)	0.11(0.087–0.15)
1	4.54(4.29–4.81)	3.59(3.15–4.04)	2.43(2.32–2.63)	0.088(0.075–0.095)
47	5.69(5.18–6.41)	3.60(3.22–3.88)	2.57(2.29–3.05)	0.079(0.069–0.093)
11	3.46(2.89–3.65)	3.55(3.06–4.21)	2.35(2.05–2.82)	0.092(0.084–0.096)
48	5.00(4.43–5.36)	3.58(3.32–4.06)	2.03(1.78–2.29)	0.081(0.078–0.091)
10	3.33(3.16–3.41)	3.95(3.63–4.26)	2.58(2.34–2.80)	0.15(0.12–0.16)
19	3.82(3.72–4.00)	4.97(4.93–5.01)	3.07(2.96–3.26)	0.17(0.15–0.19)
9	2.67(2.46–2.87)	4.68(3.95–5.26)	2.91(2.44–3.51)	0.14(0.10–0.18)
20	3.07(2.97–3.26)	5.99(5.75–6.17)	3.59(3.45–3.70)	0.17(0.14–0.22)
Adult	1.38(1.25–1.63)	6.81(5.92–7.63)	4.79(4.15–5.34)	0.13(0.094–0.15)

LITTER NO.	KIDNEYS	WOLFFIAN BODIES	ALL VISCERA
	per cent	*per cent*	*per cent*
13		12.10(11.14–12.59)	38.08
14		7.45(6.60–8.15)	34.73
24	0.22(0.17–0.30)	4.71(4.03–5.41)	34.15
17	0.69(0.39–0.97)	2.43(2.03–3.03)	30.69
21	0.65(0.60–0.69)	2.86(2.85–2.86)	32.11
8	2.59(2.35–2.89)	1.41(1.29–1.70)	36.65
2	2.54(2.23–2.91)	0.38(0.35–0.39)	27.08
5	2.19(1.74–2.69)	0.24(0.077–0.50)	27.79
16	1.76(1.66–1.79)	0.077(0.064–0.103)	20.38
23	1.52(1.21–2.65)	0.046(0.031–0.063)	17.74
4	1.77(1.67–1.85)		18.48
	0.98(0.90–1.09)		18.47
18	1.27(1.06–1.51)	0.022(0.013–0.035)	17.86
1	1.59(1.55–1.65)		18.44
47	1.40(1.27–1.55)		17.27
11	1.07(1.00–1.14)		14.81
48	1.09(0.99–1.18)		15.58
10	0.92(0.76–1.01)		15.11
19	1.24(1.06–1.51)		17.76
9	1.02(0.95–1.13)		14.39
20	1.01(0.92–1.07)		16.23
Adult	0.24(0.23–0.27)		7.85

TABLE 1 (continued)

Average percentage of net body weight (also minimum and maximum)

LITTER NO.	PANCREAS	SUPRARENALS	GONADS
	per cent	*per cent*	*per cent*
13			
14			
24			0.13(0.11–0.15)
17			M. 0.24(0.23–0.24) F. 0.076(0.061–0.091)
21	0.042(0.034–0.050)	0.11(0.11–0.11)	M. 0.25 (0.24–0.26) F.
8	0.11(0.082–0.17)	0.13(0.091–0.14)	M. 0.16 (0.15–0.17) F. 0.094(———)
2	0.081(0.078–0.086)	0.062(0.048–0.071)	M. 0.100(0.097–0.103) F. 0.077 (0.044–0.11)
5	0.12(0.059–0.16)	0.079(0.048–0.12)	M. 0.072(0.054–0.096) F. 0.047(0.036–0.061)
16	0.11(0.091–0.13)	0.035(0.027–0.040)	M. 0.084(———) F. 0.035(0.022–0.047)
23	0.12(0.082–0.14)	0.052(0.036–0.075)	M.0.059(0.044–0.082) F. 0.025(0.023–0.027)
4	0.082(0.069–0.093)	0.030(0.026–0.036)	M. 0.023 (———) F. 0.034(0.033–0.034)
3	0.11(0.093–0.13)	0.034(0.031–0.038)	M. 0.046(———) F. 0.039(0.031–0.044)
18	0.12(0.096–0.16)	0.087(0.032–0.54)	M. 0.041(0.031–0.065) F. 0.024(———)
1	0.13(0.099–0.17)	0.027(0.021–0.033)	M. 0.040(0.039–0.040) F.
47	0.13(0.10–0.15)	0.026(0.020–0.033)	M. 0.033(0.028–0.038) F. 0.023(0.021–0.026)
11	0.14(0.095–0.17)	0.019(0.014–0.021)	M. 0.038(———) F. 0.023(0.022–0.025)
48	0.14(0.12–0.16)	0.022(0.017–0.026)	M. 0.037(0.032–0.042) F. 0.019(0.015–0.023)
10	0.17(0.13–0.24)	0.016(0.014–0.021)	M. 0.028(0.025–0.033) F. 0.017(0.012–0.021)
19	0.19(0.17–0.20)	0.023(0.019–0.026)	M. F. 0.022(———)
9	0.15(0.13–0.17)	0.013(0.011–0.015)	M. 0.038(0.033–0.043) F. 0.014(0.013–0.016)
20	0.16(0.13–0.21)	0.019(0.013–0.022)	M. F. 0.035(0.025–0.046)
Adults	0.14(0.13–0.15)	0.0053(0.0032–0.0073)	M. F. 0.015(0.013–0.017)

The complete individual data on which the averages in this table are based will be deposited in The Wistar Institute of Anatomy, Philadelphia, where they will be accessible to any who may desire to use them.

The following notes apply to the preceding table. The sex was undetermined in litters 13, 14, and 24. The intestinal and stomach contents were too small for measurement in the earlier stages. In a few of the individuals in litters 5, 16, 23, 4, 3, 1, 47, 11, 10, 19, 9 and 20, and in three of the adults, the weight of the contents is estimated.

In a few other instances, the number of observations on individual organs is less than the number used of the litter. Thus, in litter 14, only two observations were made on the lungs, two on spinal cord, and three on stomach and intestines. In litter 8, only three observations were made on the thymus, and in litter 5, there were seven observations on thymus, and six on eyeballs. Litter 23 contains eight observations on eyeballs; litter 4, only one spinal cord. In the adult, only one observation each was made on the brain, eyeballs and thyroid gland; two on pancreas and suprarenals, and three on kidneys. The crown-rump length was measured on only one (the largest) adult.

From the averages of the various litters, curves of relative growth for the different organs were constructed. The percentage which the various organs form of the entire (net) body weight is used for the ordinate, and the average crown-rump length for the abscissa. For convenience in drawing these curves, certain of the litters of nearly the same size were grouped together and their averages taken. Thus, litters nos. 17 and 21 were grouped together at an average of 39 mm.; nos. 2 and 5 at 84 mm.; nos. 16, 23 and 4 at 107 mm.; nos. 3, 18 and 1 at 130 mm.; and nos. 47, 11 and 48 at 157 mm. The resulting curves are not essentially different from those in which all the litters were used individually, and the simplification due to the elimination of occasional irregularities is an advantage, particularly when several curves are combined in the same figure.

The dotted portion of the line at the right indicates the trend of postnatal relative growth, from birth to maturity. It must, of course, be borne in mind that these curves give an idea only of the general changes in the relative size of the various organs, and no information concerning the age, or the rapidity with which the changes occur.

Fig. 1 contains four curves—one each for the head, liver, and brain, and one for all the viscera taken together.

Fig. 2 contains five curves—one each for the kidneys, Wolffian bodies, heart, stomach and intestines with contents, and the same empty.

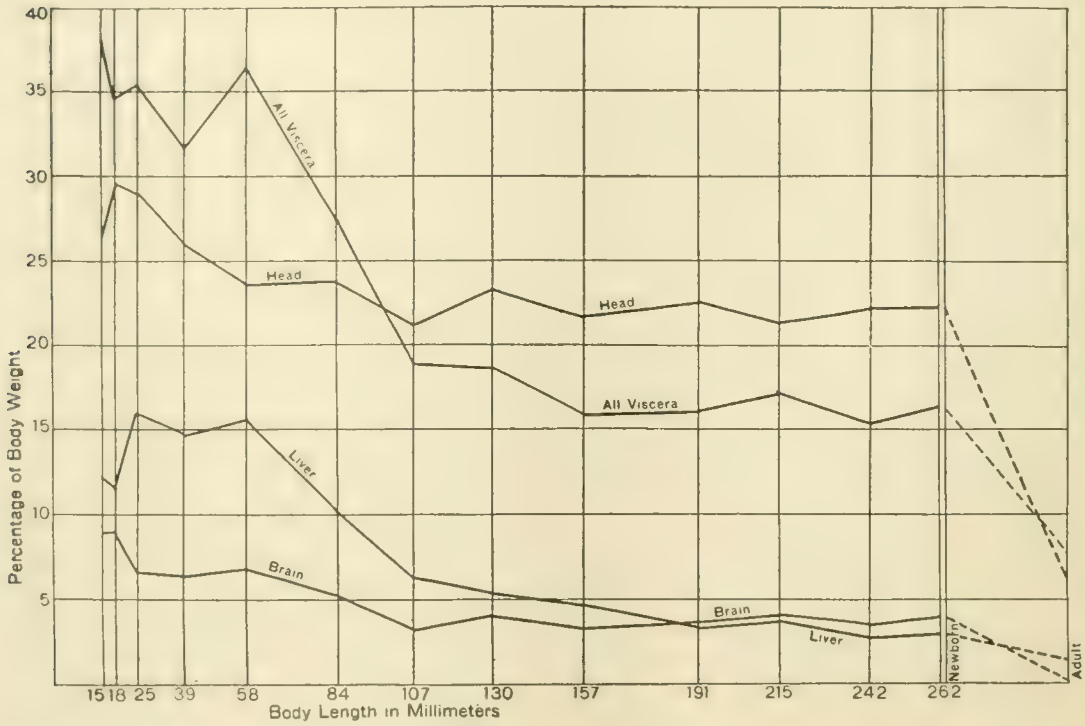

Fig. 1 Curves showing the relative growth of all the viscera, the head, liver and brain. These curves were secured by arranging the average percentage (for the litter) which the organs form of the net body weight, according to the average body length (in millimeters). In certain cases, several litters are combined, the average of the litter averages being taken (see above). The broken line at the right shows the trend of the post-natal relations, as indicated by the observations on the adults. The information thus gained is only general, as there are no indications concerning the age of the specimens, or the *rapidity* with which the changes take place.

Fig. 3 contains three curves—one each for the lungs, spinal cord, and eyeballs.

Fig. 4 includes curves for the gonads (ovaries and testes), thymus and spleen.

Fig. 5 includes curves for the suprarenal glands, thyroid and pancreas.

DISCUSSION OF THE DATA AND COMPARISON WITH GROWTH IN OTHER SPECIES

In the following pages, the body as a whole will first be considered, followed by a discussion of the viscera as a whole, and finally of each individual organ. Comparisons are made principally with the human species, for which the data are fairly complete.

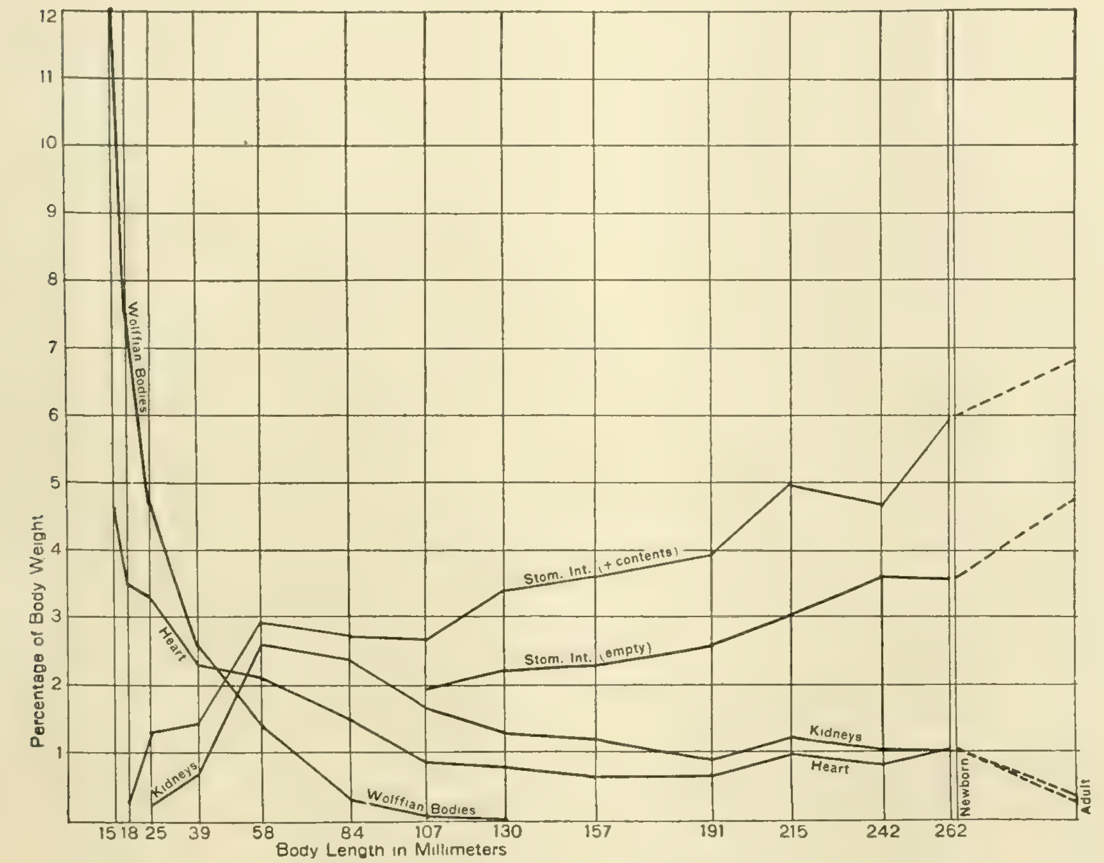

Fig. 2 Curves showing the relative growth of the Wolffian bodies, heart, kidneys, stomach and intestines with contents and stomach and intestines empty. These curves were secured by arranging the average percentage (for the litter) which the organs form of the net body weight, according to the average body length (in millimeters). In certain cases, several litters are combined, the average of the litter averages being taken (see above). The broken line at the right shows the trend of the post-natal relations, as indicated by the observations on the adults. The information thus gained is only general, as there are no indications concerning the age of the specimens, or the *rapidity* with which the changes take place.

The figures for the human embryo are quoted from Jackson ('09), unless otherwise specified. Relatively few observations are available for other animals.

1. *The body as a whole*

Table 1 gives the average body weights observed for the various litters examined, giving however, no indication of their ages. There are, however, some data available by which the age of one litter and the size at birth can be approximately estimated. From these may be roughly estimated the growth rate for the earlier and later stages.

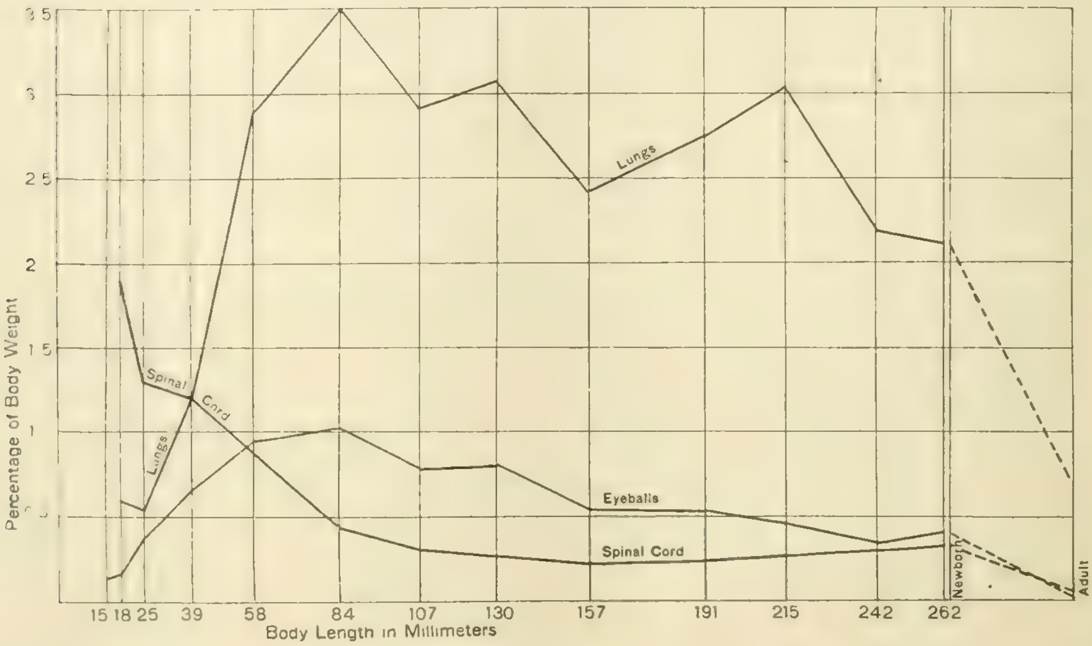

Fig. 3 Curves showing relative growth of spinal cord, lungs and eyeballs. These curves were secured by arranging the average percentage (for the litter) which the organs form of the net body weight, according to the average body length (in millimeters). In certain cases, several litters are combined, the average of the litter averages being taken (see above). The broken line at the right shows the trend of the post-natal relations, as indicated by the observations on the adults. The information thus gained is only general, as there are no indications concerning the age of the specimens, or the *rapidity* with which the changes take place.

The largest ovum found in those pig ovaries examined measured 0.177 mm. in diameter. This includes the zona pellucida which measures about 0.020 mm. in thickness (0.010 mm. counted twice in measuring the diameter). From this, the diameter of the mature ovum (including the zona pellucida) is estimated to be about 0.18 mm. The corresponding volume would be about

0.000003 cc., and (assuming the specific gravity as 1) the weight about 0.000003 g.

Keibel ('97) describes three pig embryos, aged twenty-two days, which varied from 11.7 mm. to 14 mm. in length. In the smallest litter observed by me, the average crown-rump length was 13.6 mm., and the average weight 0.25 g. (litter 13, which contained eight specimens, only three of which were dissected). These embryos would probably not be more than 23 days old, according to Keibel's figures, although some allowance must be made for variation.

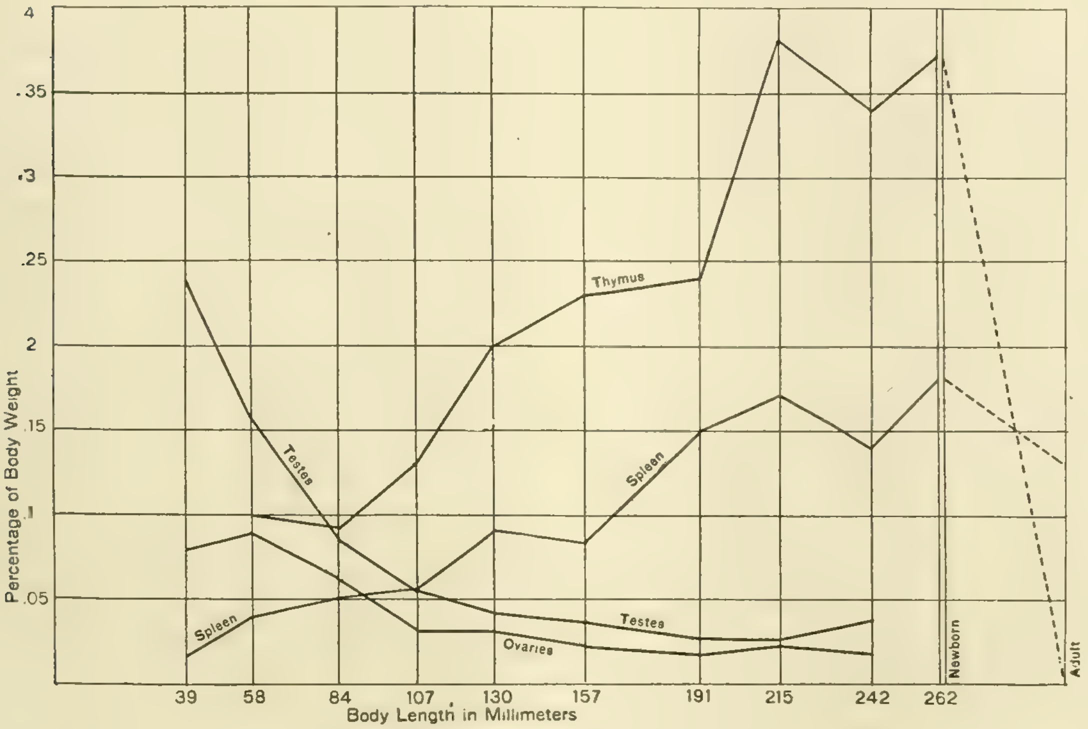

Fig. 4 Curves showing the relative growth of the testes, ovaries, spleen and thymus. These curves were secured by arranging the average percentage (for the litter) which the organs form of the net body weight, according to the average body length (in millimeters). In certain cases, several litters are combined, the average of the litter averages being taken (see above). The broken line at the right shows the trend of the post-natal relations, as indicated by the observations on the adults. The information thus gained is only general, as there are no indications concerning the age of the specimens, or the *rapidity* with which the changes take place.

The duration of pregnancy in the pig is given by Coburn ('94) as 112 days, by Long ('06) as usually 112, varying from 110 to

116 days according to the age of the mother, and by Spencer ('98) as 16 weeks. This would place the usual time as 16 weeks or 112 days.

Long ('06) gives some figures for the weight of litters from one year old sows. The litters average 7.8 pigs to the litter, the total average weight being about 14.2 lbs, or about 6,442 gms. This is an average weight of about 826 g. per pig. For sows two to three years old, the pigs average about 1,190 g. each. For those about five years old, the pigs are still larger, weighing about 1300 g. each on the average (individual data not given).

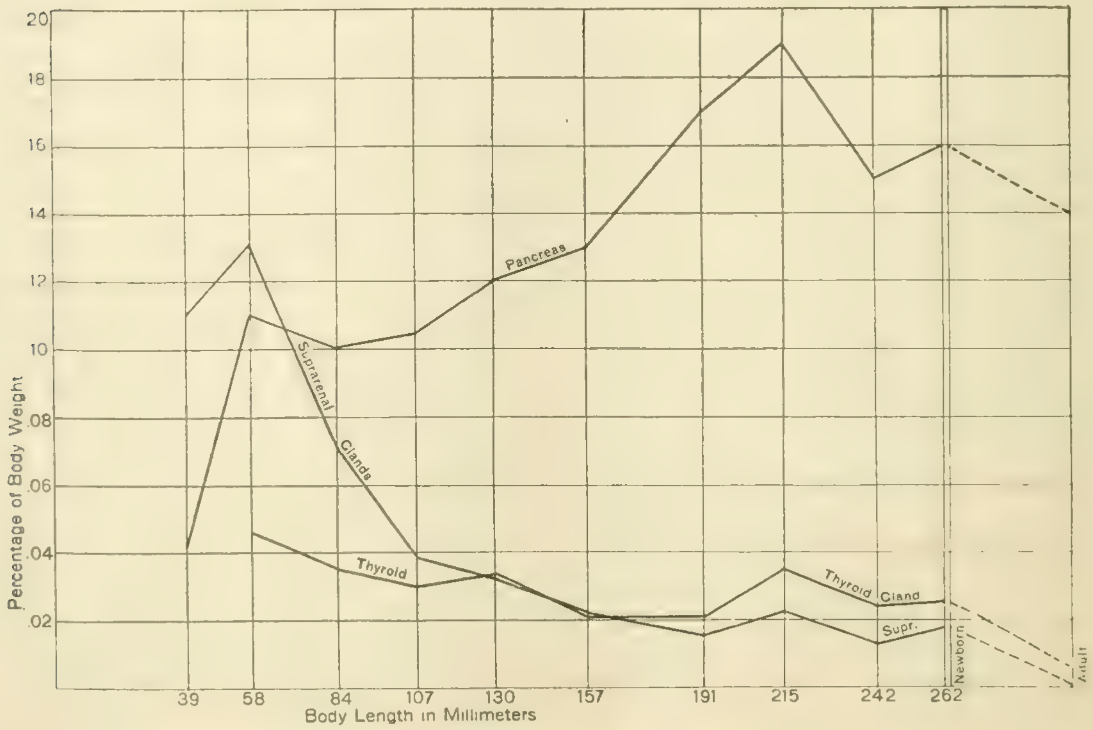

Fig. 5 Curves showing the relative growth of the pancreas, thyroid and suprarenal glands. These curves were secured by arranging the average percentage (for the litter) which the organs form of the net body weight, according to the average body length (in millimeters). In certain cases, several litters are combined, the average of the litter averages being taken (see above). The broken line at the right shows the trend of the post-natal relations, as indicated by the observations on the adults. The information thus gained is only general, as there are no indications concerning the age of the specimens, or the *rapidity* with which the changes take place.

As the hogs which go to market are usually about ten to twelve months old, the average weight of the full term fetus from such animals would therefore be approximately 826 g. The oldest litter examined in my observations averaged 745 g. These pigs are therefore probably well along in the last month of fetal life.

During the 23 days immediately succeeding fertilization, the weight thus apparently increases to about 0.25 g., an actual increase of about 83,000 times the weight of the ovum. Even this figure is too small, as a part of the ovum goes to form the membranes, etc. But this gives an idea of the enormously rapid growth rate in the early stages. For the whole ensuing fetal period of 99 days, the fetus increases in actual weight from 0.25 g. to 826 g., or about 3300 times the weight at 23 days. That is, in a period about four times as long, the increase is only about one twenty-fifth as great. If the early rate of increase were maintained throughout the fetal period, the newborn animal would weigh about 12 million billion grams!

The weight at birth, 826 g., represents an increase of over 275 million times the weight of the ovum.

The four adult hogs examined (age about twelve months) averaged about 104.5 kg. in weight. We may safely assume the average young adult weight to be about 100 kg. The increase from a weight at birth of 826 g. to an adult weight of 100 kg. represents an increase of only 121 times. This is certainly an enormous decrease in the relative growth rate, even when compared with the last 99 days of prenatal life. The total increase from the weight of the ovum to the weight of the one year adult (sixteen months prenatal and postnatal time) amounts to about thirty-three billion times the weight of the ovum.

Although these data are limited in extent, they show such enormous differences for the various periods that we may safely assume the conclusions of Muehlmann ('00), strongly emphasized by Minot ('07) and Jackson ('09), to be true also for the pig; that is, that growth takes place most rapidly in the earliest stages, the rate decreasing, at first rapidly, then more slowly, throughout prenatal and postnatal life.

In table 2 some data are given for comparison of growth in the pig, human, rabbit, white rat and chick. The figures for the rabbit are derived from Waldeyer ('06), Fehling ('77) and Minot ('07); those for the white rat from Donaldson ('06) and unpublished observations by Jackson; those for the chick from Welcker ('03) and Davenport ('08).

TABLE 2

Table of comparative growth

ANIMAL	OVUM	THREE WEEKS	
	Absolute weight	Absolute weight	Number of times increase
	g.	g.	
Pig	0.000003	0.25	83,000
Human	0.000004	0.016	4,000
Rabbit	0.000003	11.7	3,900,000
White rat	0.000003(?)	5.0	1,670,000
Chick	(total egg) ? (35–40 g.)	30.0	(?)

ANIMAL	112 DAYS		SIXTEEN MONTHS (PRE + POSTNATAL)	
	Absolute weight	Number of times weight of ovum	Absolute weight	Number of times weight of ovum
	g.		g.	
Pig	826	275,000,000	100,000	33,000,000,000
Human	120	30,000,000	8,000	2,000,000,000
Rabbit	1000 (?)	333,000,000(?)	2,000(?)	667,000,000(?)
White rat	140	47,000,000	275(?)	92,000,000(?)
Chick	1000 (?)	(?)	2,000(?)	(?)

Conclusions on comparative growth

From table 2 of the development of these various animals, it will be seen that the general course of relative growth of the pig agrees with that of all the others, in that the rate is very rapid in the early embryonic stages, decreasing at first rapidly, then more slowly, throughout prenatal and postnatal life, until the adult size is reached.

The rapidity of growth, however, is quite different in the various animals at corresponding periods. Thus, at the end of three weeks of prenatal life, the pig has increased about twenty times as rapidly as has the human embryo, but only about one-twentieth as rapidly as the white rat and one-fiftieth as rapidly as the rabbit. After birth of the rat and rabbit, however, their growth rate is greatly decreased; so that at 112 days the pig (newborn) has far outstripped the rat (275 million to 47 million), and is approaching the rabbit, which has reached a weight of about 333 million times the weight of the ovum. The human embryo still lags behind (30 million) but is approaching the rat. At one year postnatal (sixteen months total) the pig has reached 33 billion times the weight of the ovum. Next in order is the human (seven months old) with an increase of two billion times, while the rabbit and white rat have dropped far behind. Thus the animals with a short gestation period (rabbit, rat) have a more rapid growth rate during that period, but are thereafter overtaken and outstripped by those with a longer gestation period (pig, human).

At the time of birth, the growth rate always undergoes a rapid decrease, the prenatal rate always being far more rapid than the postnatal. There seems to be no definite relation, however, between the length of the gestation period and the ratio of newborn to adult weight. Of the five species considered, the human is relatively nearer the adult weight at birth, the ratio being about 1:19. The pig is farthest from the adult, the ratio being about 1:120. The rabbit, rat and chick take an intermediate position, their ratio being about 1:60 (table 3).

TABLE 3

Ratio of adult to newborn weight

ANIMAL	WEIGHT OF NEWBORN	WEIGHT OF ADULT	$\frac{\text{WEIGHT ADULT}}{\text{WEIGHT NEWBORN}}$
	g.	g.	
Pig	826	100,000	121
Human	3200	60,000	19
Rabbit	38.35	2,500 (?)	65(?)
White rat	5.00	300	60
Chick	30.00	2,000	66(?)

2. *Relative growth of the viscera*

Under this heading will be considered the relative growth of all the viscera together (including the brain and spinal cord, and excluding the contents of the stomach and intestines) which were measured. In a negative way, this gives also the relative growth of the other structures of the body, chiefly skeleton, musculature and skin.

A reference to fig. 1 will show the general trend of the curve. The maximum relative size occurs in the 15 mm. litter, where all the visceral organs form 38.08 per cent of the total body. At 58 mm. they form 36.65 per cent, and in the late fetus, 16.23 per cent of the total weight. In the adult, they form only 7.85 per cent of the total weight.

From this it is evident that the skeleton and musculature are relatively small at first, increasing at first rapidly, then more gradually, until, in the adult, they form about one half more relatively than they did at the fetal maximum for the organs. This is based on the assumption that there is relatively little change in the relative weight of the skin, etc.

Corresponding figures for the human show that the viscera form about 36 per cent of the total body in the second fetal month, slowly decreasing to about 26 per cent at birth, reaching about 13 per cent in the adult.

The total relative weight of the viscera is apparently about the same for the pig and human in early fetal life; but at birth that for the pig is much below the human. The decrease for the viscera and the increase in relative size of the remaining structures are very marked between birth and maturity, both in the pig and the human.

According to Jackson, the brain and spinal cord in the human embryo, at the beginning of the second month, have nearly three times the volume of the organs lying ventral to the body axis; at birth they are about equal; while in the adult, the ventral organs are six times as large as the brain and spinal cord. In the pig, the nervous system is relatively much smaller. In the 18 mm. litter, the ventral organs are nearly three times as large as the brain and cord: at birth they are more than three times as large; while in the adult, they are about ninety times as large.

3. *Relative growth of the various organs*

The relative growth of the organs together having been shown, it is now necessary to take up the growth of each individual organ separately, as their growth is by no means uniform.

The head. Table 1 and the head curve in fig. 1 show that the head was found relatively largest in the second stage examined (18 mm.), forming on the average, 29.69 per cent of the total. Throughout the earlier fetal stages it forms from 20 per cent to 28 per cent of the total, and in the later averages about 22 per cent to 23 per cent. Its minimum observed relative size is 19.65 per cent in the 108 mm. litter. In the nearly full term fetus it averages 22.3 per cent of the total. In the adult it forms 6.26 per cent of the total body weight. The decrease in the relative size of the brain is much larger than that of the entire head, so that the facial structures form an increasingly larger portion of the head in the later fetuses and the adult.

In the human, the head reaches a maximum relative size of 45 per cent during the second month. It then decreases gradually in relative size, forming about 26 per cent of the total at birth. The adult human head forms 6 to 9 per cent of the total body weight.

The head of the pig at no stage observed reaches as large a relative size as does the human at a corresponding stage, having, for the most part, a relative size about two-thirds that of the human. In the adult, the relative size is more nearly equal in the two species.

In all vertebrates, from the fishes upward, the embryonic head is relatively large, especially in the early stages. The extent to which this is true varies in the different forms, the head being in general best developed in the amniota. It is perhaps largest in bird embryos, where it may form more than half of the entire body (chick). There is also much variation in different species of mammals, as shown by the difference between pig and human.

Brain. (*Table 1: fig. 1*). The maximum relative size of the brain (table 1) was found in the earliest stages examined (15 mm. and 18 mm., accompanying the maximum relative sizes of the head). It then forms, on the average, nearly 9 per cent of the total body weight. The relative size then decreases, rapidly in the earlier stages, then more slowly. In the fetus nearly full term, it forms nearly 4 per cent of the total, and in the adult, 0.087 per cent.

A comparison of the relative size of the head and brain shows that, in the first stage examined, the brain forms about one-third of the head, at 86 mm. about one-fifth, and throughout the later part of fetal life and at birth, about one-sixth. In the adult it forms only about one-seventieth of the head.

This is a very small relative size when compared with the human, both in prenatal and postnatal stages. The maximum relative size for the human brain occurs during the second month (at the same time as for the head), when the brain forms about 20 per cent of the entire body. This is about one half of the entire head. At birth, the brain forms about 12.8 per cent or 14.6 per cent (still-born or live-born), which is about one-half of the entire head. Vierordt estimates the adult human brain at 2.16 per cent of the total body, forming about one-third to one-fourth of the head.

According to figures from Donaldson ('08), the brain in the newborn white rat forms about 5 per cent of the whole body, which is about one-fourth larger relatively than in the pig at birth. In the adult rats the brain forms about 0.7 per cent of the body, which is eight times as large relatively as in the adult hog.

In the dog-fish data by Kellicott ('08), show that at birth the brain forms 1.11 per cent of the total, while in the adult it forms only 0.085 per cent or about the same relatively as in the pig.

Some observations on the chick, recorded by Welcker and Brandt ('03), indicate that, at the ninth day of incubation, the brain forms 28.2 per cent of the body; newly hatched, 3 per cent; adult, less than 0.5 per cent.

Spinal cord. (*Table 1: fig. 3*). The spinal cord in the pig embryo has its maximum relative weight (of the stages observed) at 18 mm., where it forms 1.87 per cent of the total. Decreasing, at first rapidly, then more slowly, at 156 mm. it reaches its minimum prenatal relative size, about 0.21 per cent of the total. Throughout the remainder of the stages examined, it averages about 0.25 per cent. In the late fetus, however, it forms 0.33 per cent, while in the adult it forms 0.043 per cent of the total.

In the first stage observed, its weight is about one-fifth that of the brain, later in the fetal period, about one-twelfth to one-fifteenth, in the late fetus reaching one-twelfth. In the adult the weight of the cord is about one-half that of the brain. Its relative growth rate must be much greater in postnatal life than is that of the brain; or, otherwise expressed, the decrease in relative size of the brain is much greater than that of the cord.

The cord is relatively larger in the human fetus than in the pig in the earlier stages, but smaller in the later stages. Its maximum in the human embryo is 4.85 per cent in the fifth week. At 17 mm., it forms 3.43 per cent, in comparison with 1.87 per cent at 18 mm. in the pig. At birth, the human cord forms about 0.15 per cent of the total weight, which is only one-half the relative size of the pig's cord. In the human adult, the cord forms 0.06 per cent of the total (Vierordt), which is almost twice that of the pig. The conditions regarding comparative prenatal and postnatal growth of brain and cord are similar in both human and pig; that is, the brain has the more rapid relative growth rate in prenatal life. In postnatal life the relative growth rate of the cord is more rapid, as Donaldson has pointed out for the human.

Figures from Donaldson ('08) show that at birth the spinal cord in the white rat forms, on the average, 0.73 per cent of the total, while in the adult (10–12 months), it forms about 0.20 per cent. The decrease is therefore similar to that in the pig and human. Data by Welcker indicate a similar decrease for the chick, but not for the dog.

The eyeballs. (*Table 1: fig. 3*). The eyeballs, starting with an average relative size of 0.14 per cent of the total at 15 mm., increase rapidly to a maximum of 1.15 per cent at 86 mm. From this stage, they decrease slowly until, at birth, they form 0.41 per cent of the total. In the adult they form only 0.011 per cent of the total weight.

From a few scattering observations on the human eyeballs, it seems that they are relatively smaller than those of the pig in the early stages; of about the same relative size in the later stages of prenatal life; relatively smaller at birth, and about twice as large relatively in the adult.

Welcker and Brandt record some observations on the chick embryo. At the eleventh day of incubation, the eyeballs form nearly 25 per cent of the total body. At birth they form about 3 per cent, and in the adult about 0.3 per cent.

Thyroid gland. (*Table 1: fig. 5*). The thyroid makes its first appearance (being too small for accurate dissecting earlier) in the 58 mm. litter, when it has its maximum observed relative size of 0.045 per cent of the entire body weight. From then on until the end of the prenatal period, it averages from 0.02 per cent to 0.03 per cent, forming 0.026 per cent, at birth. In the adult it forms only 0.004 per cent of the total. The individual measurements are rather variable for this gland, but it averages very regularly throughout.

The human thyroid, forming 0.035 per cent of the total embryo at two months, increases to 0.11 per cent or 0.125 per cent (stillborn or live-born). In the adult, it forms 0.05 per cent of the total according to Vierordt. It is, therefore, at all stages, a relatively larger and probably more important organ in the human than in the pig.

Thymus. (*Table 1: fig. 4*). The thymus is liable to a considerable amount of individual variation, and shows a gradual increase in relative size throughout prenatal life. Where first measured, at 58 mm., it forms practically 0.1 per cent of the total weight. Near full term, it forms 0.37 per cent of the total. It was not found in the adult, though it may possibly have been overlooked.

In the human, the thymus forms 0.008 per cent of the entire body at the end of the second fetal month; and about 0.3 per cent at birth. Vierordt gives the adult size as 0.04 per cent of the total weight.

In general, the relative size and course of growth of the thymus appears somewhat similar in the pig and in the human.

The lungs. (*Table 1: fig. 3*). The lungs are considered together because, while there is a difference in size, their ratio to each other is fairly constant. The curve for both lungs together is given in fig. 3. Forming 0.6 per cent of the total weight at 18 mm., the lungs increase rapidly in relative size until, at 86 mm., they form 3.89 per cent of the total body weight. From here the decrease in relative size is somewhat irregular to the late fetus, where they form about 2 per cent of the total weight. In the adult they form about 0.69 per cent. In the smallest litters examined, there is apparently no appreciable difference in size between the two lungs. The difference between the two lungs in the later stages, while holding fairly constantly to the ratio (right to left) of 7:5, is liable to considerable individual variation, as is the relative size of each lung.

Approximately the same course of growth occurs in the human. There is an initial rise, the maximum relative size occurring in the fourth fetal month, when the lungs average 3.29 per cent of the total weight. In the still-born they average 1.7 per cent; in the live-born, 2.18 per cent. In the adult they form 1.5 per cent of the total (Vierordt). In relative size therefore, the human lungs are approximately equal to those of the pig during prenatal life, but are about twice as large in the adult. In both, the maximum occurs early in prenatal life.

The right lung in the human is also larger than the left, averaging about 20 per cent larger. In the adult, the right lung is usually stated to be only 10 per cent larger than the left. In the pig, however, the right lung averages about 40 per cent greater than the left, both in the fetus and the adult.

The heart. (*Table 1: fig. 2*). The heart has its greatest observed relative size in the earliest stage examined (15 mm.), forming 4.64 per cent of the total weight. An examination of earlier stages would possibly show a still larger maximum. It decreases in relative size rapidly at first, then more slowly, until at 125 mm. it forms only 0.66 per cent of the total body weight. In the later fetuses there is a gradual rise, the heart forming 1.03

per cent of the total weight in the 26 cm. fetus (nearly full term). In the adult it forms 0.32 per cent, about one-third that of the late fetus.

In the human the heart also has its maximum early, being estimated at more than 5 per cent in a four-weeks embryo; 3.64 per cent in the fifth week (11 mm.) and 0.85 per cent in the third month. At birth, it forms 0.7 per cent or 0.77 per cent (still-born or live-born). Vierordt estimates the adult human heart to form 0.56 per cent of the total weight.

The heart is therefore similar in relative size in pig and human during fetal life. It appears, however, to be relatively smaller at birth, and larger in the adult, in the human.

Stomach and intestines. (*Table 1: fig. 2*). The stomach and intestines (including mesentery) differ from all other organs observed, in that they increase greatly in relative size in the adult as compared with the fetus. This is the case whether they are considered with or without contents.

At 18 mm. they form 0.26 per cent of the total body weight, after which they increase, at first very rapidly, then more slowly (and with considerable individual variation) to a maximum (for prenatal life) of 6 per cent in the late fetus. In the adult they form 6.81 per cent of the total weight. These figures are for stomach and intestines plus contents, which, especially in the later stages, present a great deal of variation.

A better index of the growth is shown by the figures for the stomach and intestines without contents. In the stages up to and including the 42 mm. stage, there would seem to be no appreciable contents, and here the stomach and intestines amount to 1.62 per cent of the body weight. These organs gradually increase throughout fetal life, forming at 26 cm. about 3.59 per cent of the total body weight, being about 40 per cent less than the same with contents (5.99 per cent). In the adult they form (empty) about 4.79 per cent of the net body weight, about 30 per cent less than the tract with contents.

The range of variation is large, due to the extreme variations in contents, but the empty tract is, in later prenatal life, about 35 to 40 per cent less in weight than the tract with contents.

In the human, the intestinal tract is relatively small in the early stages, increasing rapidly to full term, when the empty tract forms 1.03 per cent of the total weight. The contents here are about twice as great as the empty intestinal tract. Vierordt estimates 2.06 per cent of the total body weight for the empty adult tract.

The empty intestinal tract of the pig is therefore relatively much larger than that of the human, both in the later fetal stages and in the adult, while the contents are apparently relatively smaller.

The liver. (*Table 1: fig. 1*). In the earliest stage examined (15 mm.), the liver forms 12.25 per cent of the total body weight. In the next succeeding stages it forms the most prominent organ. It has an early maximum, reaching 15.88 per cent of the net body weight in the 25 mm. stage and remaining at about this relative size until the 58 mm. stage. From here it decreases sharply, forming 5.01 per cent at 109 mm. In the late fetus it forms 3. per cent of the total (net) body weight. In the adult it forms only about 1.38 per cent of the total body weight. The maximum relative weight may be somewhat more than 15.88 per cent, and come just preceding or following the 25 mm. stage.

In the human fetus, the liver never reaches more than 7.5 to 10 per cent of the total body, and this maximum size occurs during the second and third months. During the fourth month, the liver drops to an average of about 5 per cent, which it maintains throughout fetal life, averaging 5.23 per cent in live-born infant. Vierordt gives the average for the adult human at 2.75 per cent. Therefore, in the earlier fetal stages, the liver has a much greater relative size in the pig than in the human, but in the later fetal stages and in the adult, it is relatively much smaller.

As is the case in the pig and human, the liver is much smaller in the adult than in the embryo or new born in the chick, dog, stickleback and shrew (Welcker). But it is relatively larger in the adult for the salamander (Welcker) and dog-fish (Kellicott).

Spleen. (*Table 1: fig. 4*). The spleen is liable to very great individual variations in relative size. First observed in the 42 mm. litter, it there has a relative size of 0.016 per cent of the total weight. It gradually increases in relative size, until, in the 26 cm.

fetus, it forms on the average 0.17 per cent of the total weight. In the adult, it forms about 0.13 per cent.

Practically the same course of growth is found in the human, although here the spleen is relatively larger than in the pig. The maximum is over 0.4 per cent in the 8th and 9th fetal months. It forms 0.43 per cent in live-born infants, and 0.25 per cent for the adult (Vierordt). The individual variations would seem to be greater in the human than in the pig.

Pancreas. (*Table 1: fig. 5*). Starting with a relative size of 0.042 per cent of the total body weight at 42 mm., where it was first observed, the pancreas increases, at first rapidly, then more slowly to a maximum of 0.19 per cent at the 215 mm. stage. At 26 cm., it forms 0.16 per cent of the total weight; in the adult, 0.14 per cent. In the pig, the variations in individual size appear very great, due perhaps in part to difficulty in dissecting it out perfectly. During the greater part of fetal life, the pancreas averages about 0.1 per cent to 0.13 per cent of the total weight.

Judging from the data available for comparison, the pancreas is slightly heavier relatively in the pig than in the human throughout the greater portion of fetal life, and has about the same relative size in the adult.

Suprarenal glands. (*Table 1: fig. 5*). At 25 mm., the suprarenal glands are readily visible with the dissecting lens, but, owing to their position and attachments, cannot be readily dissected out and weighed.

At 42 mm., the suprarenal glands form about 0.11 per cent of the total weight. At 58 mm., they form 0.13 per cent of the total, which is the maximum observed relative size. Throughout the later part of fetal life, they average about 0.015 to 0.025 per cent of the total, forming 0.019 per cent in the latest stage examined. In the adult they are very small organs, forming about 0.005 per cent of the total. There is no marked difference between the right and left in size.

In the human fetus, the left suprarenal is usually the larger of the two. During the second month, the suprarenals form about 0.3 per cent of the total body volume, increasing to a maximum of 0.46 per cent in the third month. At full term they form about

0.24 per cent. In the adult, they form 0.01 per cent (Vierordt). The suprarenals, therefore, have a much larger relative size in the human than in the pig, both in prenatal and postnatal life.

Gonads. (*Table 1: fig. 4*). The gonads were first accessible by dissection at 25 mm., when they average 0.13 per cent of the total weight (sex not determined). From this point on, there is considerable difference in the two sexes, the testes (including epididymis) averaging heavier relatively than the ovaries. Rising rapidly to a maximum, the testes form 0.24 per cent at 42 mm., decreasing to 0.038 per cent at 242 mm. At 58 mm. the ovaries have their maximum size of 0.094 per cent of the total, decreasing to 0.014 per cent at 242 mm. At 26 cm. the ovaries (average of two observations) form 0.035 per cent of the total body weight. This is probably either erroneous, or an abnormal condition, hence the curve in fig. 4 was not extended to this point. In the adult (2 F.) the ovaries form 0.015 per cent of the total body weight. (No data for males).

A similar relation is also found in the human, the gonads being relatively larger in the earlier part of the prenatal growth and the testes being relatively heavier than the ovaries. In a human embryo of the fifth week (11 mm.), the anlages for the gonads (sex undetermined) form 0.085 per cent of the total body volume. In the later fetal stages, the human testes are about twice as large relatively as the ovaries, a relation similar to that in the pig.

Kidneys and Wolffian bodies. The kidneys (table 1; fig. 2) were first measured at the 25 mm. stage, where they average 0.22 per cent of the total body weight. They increase rapidly to a maximum relative size of 2.59 per cent of the total weight at 58 mm. At 191 mm. they form 0.92 per cent, which is the minimum for later fetal life. Near full term they form 1.01 per cent of the total, and in the adult, 0.24 per cent. There is apparently no constant difference in size between the right and left.

In the human, the kidneys form about 1 per cent in the later fetal stages. Vierordt gives 0.46 per cent as the relative size in the adult. The human kidneys first appear in the second month, and from that time onward the left kidney is quite usually slightly larger than the right.

It would appear from this that the kidneys, throughout the later part of fetal life, have about the same relative size in the pig and human, but are larger relatively in the human adult.

Agreeing with the pig and human, the kidneys appear relatively smaller in the adult than in the embryo shrew, dog and chick; but they are relatively larger in the adult stickleback and salamander (Welcker and Brandt).

The Wolffian bodies (table 1: fig. 2) of the pig have their maximum very early in fetal life, probably at an earlier stage than can be readily dissected. At 15 mm. they have by far the largest relative size observed,—12.1 per cent of the total weight. From this stage the decrease is rapid. In the 18 mm. pigs they form 7.45 per cent; 25 mm., 4.71 per cent; 37 mm., 2.43 per cent; disappearing about 125 mm.

The Wolffian bodies present a very interesting case, as they are the only structures observed (except postnatal thymus?) in which a decrease in absolute weight takes place.

The following table expresses the relations for this organ:

TABLE 4

Absolute and relative weight of the Wolffian bodies

LITTER	AVERAGE WEIGHT WOLFFIAN BODIES	AVERAGE PER CENT OF TOTAL BODY
	g.	
13 (15 mm.)	0.0335	12.10
14 (18 mm.)	0.0522	7.45
21 (25 mm.)	0.080	4.71
8 (58 mm.)	0.146	1.41
2 (82 mm.)	0.105	0.38
16 (108 mm.)	0.057	0.077
18 (125 mm.)	0.029	0.022

Corresponding to the decrease in relative size of the Wolffian bodies, there is, as might be expected, an increase in the size of the kidneys. The first record of the kidneys (0.22 per cent) corresponds to a size of 4.71 per cent for the Wolffian bodies. The maximum relative size of the kidneys (2.59 per cent) corresponds to a size of 1.41 per cent for the Wolffian bodies. At the time of disappearance of the Wolffian bodies, the kidneys form 1.27 per cent of the total weight. Shortly after this, the kidneys increase to 1.59 per cent (table 1; fig. 2).

In the human, the Wolffian bodies form 0.6 per cent of the total volume in an embryo of the fifth week (11 mm.), in which the renal anlages are just appearing. From this time onward, they become both absolutely and relatively smaller, just as in the pig, disappearing about the beginning of the third month (31 mm.). The Wolffian bodies in the pig are therefore relatively much larger than in the human embryo, and persist for a longer time.

Differences according to sex

The data from the various litters were sorted out and grouped according to sex, the averages being calculated for each sex in each litter. The results were of little value, however, on account of the small number of specimens, only nine litters having two or more of each sex. Although it was therefore not thought necessary to reproduce this table, certain indications which it showed are worthy of mention.

In the first place, the total body weight almost constantly averages higher in males than in females of the same litter. It is known that the human males average heavier in weight than the females of the same age in prenatal life and at birth.

The liver in the pig fetus was found relatively heavier in the female in most cases where a comparison was possible. This is also almost constantly true in the human species.

The gonads, beginning with the time when the sexes are first distinguishable are found relatively heavier in the male than in the female pig, as already mentioned. This is also in agreement with the human species.

For other organs, no definite conclusions concerning sexual differences can be drawn, and even those mentioned rest upon a very uncertain basis. Many more observations are necessary, in order to distinguish variations due to sex from accidental variations.

SUMMARY

The general conclusions concerning the growth of the pig may be summarized as follows:

1. The weight of the ovum is about 0.000003 g. The increase in weight is at least 83,000 times during the first 23 days. At the end of 112 days (full term) the increase is 275 million. The weight of the (young) adult hog is about 333 billion times that of the ovum. Comparative figures given for the human, rabbit, rat and chick show that all these forms agree with the general law that the rate of growth is by far most rapid at the beginning of prenatal life, decreasing at first rapidly, then more slowly, throughout prenatal and postnatal life.

2. The viscera of the pig embryo (including the brain and spinal cord) taken all together have their maximum relative size, about 38 per cent of the whole body, in the earliest stage examined (15 mm.), decreasing to about 16 per cent at birth. In the adult, they form nearly 8 per cent of the whole body.

3. The head attains its maximum observed relative size at 18 mm. forming nearly 30 per cent of the total, decreasing to about 22 per cent at birth, and about 6 per cent in the adult. It is always relatively smaller than the human head at corresponding stages.

4. The brain also attains its maximum observed relative size, at about the same time as the head, forming about 9 per cent of the total and about one-third of the head. In later stages it decreases until near full term it forms almost 4 per cent of the total, and about one-sixth of the head. In the adult it forms about 0.087 per cent of the body, and about one-seventieth of the head. The brain of the pig is at all stages relatively much smaller than that of the human.

5. The spinal cord has its maximum observed relative size 1.87 per cent of total body, at 18 mm. (probably larger in earlier stages), decreasing at first rapidly, then more slowly, and forming 0.33 per cent of the body at birth. In the adult, the cord forms about 0.04 per cent of the body.

6. The eyeballs attain their maximum observed relative size at 86 mm., forming 1.15 per cent of the body; decreasing to 0.4

per cent near birth. In the adult, they form only 0.011 per cent of the body.

7. The heart forms 4.64 per cent of the body weight at 15 mm., decreasing to about 1 per cent near birth, and averaging about 0.75 per cent during the greater number of the prenatal stages examined. In the adult hog, the heart averages 0.32 per cent of the body weight.

8. The lungs increase to a maximum of 3.9 per cent of the total body weight at 86 mm., decreasing irregularly thereafter to about 2 per cent near birth. The right lung is larger than the left in the approximate ratio of 7 to 5. The lungs in the adult form only about 0.7 per cent of the body.

9. The liver increases to a maximum relative size of 15.88 per cent of the body weight at 25 mm., decreasing to 3.1 per cent near birth. Its maximum relative size is over twice as large as that of the human. In the adult the liver of the pig averages 1.38 per cent, being only about half that of the human.

10. The kidneys increase rapidly to a maximum relative size of 2.59 per cent of the body weight at 58 mm., decreasing thereafter to an average of 1.01 per cent near birth; forming about 0.25 per cent in the adult. The Wolffian bodies are at first relatively enormous, forming over 12 per cent of the body weight at 15 mm. They decrease rapidly, however, in relative (and, after 58 mm., in absolute) size.

11. The spleen, pancreas and thymus increase gradually from the beginning, averaging about 0.17 per cent, 0.16 per cent, and 0.37 per cent respectively, of the total body weight near the end of fetal life. In the adult the figures are 0.13 per cent and 0.14 per cent for spleen and pancreas respectively.

12. The thyroid gland after 58 mm. decreases slightly in relative size throughout prenatal growth, averaging about 0.026 per cent of the body at the close of the fetal period, and 0.004 per cent in the adult.

13. The suprarenals form about 0.13 per cent of the body at 58 mm., decreasing thereafter to about 0.019 per cent near birth, and about 0.005 per cent in the adult.

14. The stomach and intestines increase gradually throughout the prenatal period, forming near full term about 6 per cent with contents, or 3.6 per cent empty. In the adult, they increase to 4.79 per cent empty or 6.8 per cent with contents.

15. During prenatal life the total weight of the body and the relative weight of the gonads are greater in the male, while the relative weight of the liver is usually greater in the female.

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THE MATURATION OF THE EGG AND OVULATION IN THE DOMESTIC CAT

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From the Sheffield Biological Laboratory of Yale University

THIRTEEN FIGURES

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INTRODUCTION

Knowledge of the essential nature of the maturation of the germ cells, of fertilization and of cleavage was won chiefly from the study of invertebrates, although Van Beneden ('75), studying the rabbit, upon some points anticipated the brilliant results of the researches of Oscar Hertwig ('75–'78).

Employing improved cytological methods and with very abundant material for study, Sobotta ('95) published in full the results of an investigation upon the egg of the mouse, which established a point of departure for researches upon the development of the mammalian egg undertaken since that time. Papers by Kirkham ('07) and Lams and Doorme ('07) have led Sobotta himself to modify some of the views which he held concerning polar body formation in the mouse egg. He no longer believes that only 10 per cent of the mature eggs of this animal form two polar bodies, but agrees that its maturation processes conform to the usual type.

Other important papers upon the early development of mammalian eggs have been published by Rubaschkin ('05) upon the guinea pig and O. Van der Stricht ('09) upon the bat. The last paper to appear upon this subject is by Sobotta and Burckhard ('10) entitled "Reifung und Befruchtung des Eies der Weissen Ratte." In short, maturation has been studied thoroughly and the results of the study presented in a conclusive manner in the case of the bat, guinea pig, mouse and rat only.

On account of the difficulties encountered in procuring the eggs of representatives of the higher orders of mammals their study has been very much neglected. These difficulties consist in the less frequent periods of oestrus, the smaller number of eggs discharged at one ovulation, the longer period of gestation and the greater difficulty in keeping the animals and breeding them in captivity. In addition by no means the least obstacle lies in the larger size of the ovaries which must be prepared for examination. Restricted by these conditions, knowledge of the maturation of the eggs of mammals higher than rodents is confined to that contained in preliminary reports by R. Van der Stricht ('08) and O. Van der Stricht ('08) upon the cat and dog respectively and to scattered notes upon maturation distributed sparsely through a great mass of literature devoted to other problems.

In addition to describing the morphological changes in the cat's egg during maturation the present paper indicates an unusual physiological condition connected with the process in this animal.

THE EGG OF THE CAT

Material and methods

Although some of the material upon which this study was made was furnished by animals in which the sequence of events during the period of oestrus was incompletely known, practically all of importance was obtained from individuals which had been for a variable time under observation in captivity. It was found to be quite possible to carry on breeding operations in the laboratory, although Winiwarter and Saintmont ('08) state that cats will not breed under restraint. It is only fair to say, however, that there is great variation in the behavior of males and that, while observers were present, few of those tried would act promptly, or at all. In a considerable number only one was found which could be relied upon to afford a final test for oestrus. This cat would immediately cover, or attempt to cover, any female put in with him, and her willingness or unwillingness to allow copulation settled the doubtful point.

There is little difficulty in deciding when copulation has been effected. Occasionally, however, one finds a female, usually young, which does not give the customary response. In such cases a portion of the contents of the vagina was withdrawn with a fine pipette, and submitted to microscopic examination for spermatozoa, which, when found under such conditions, exhibited great activity.

The ovaries of all animals killed were examined at once to determine whether ovulation had or had not occurred. Each recently ruptured follicle was marked, by an opening, the edges of which were generally, but not always, red, rough and swollen to form a characteristic elevation. Within the space of three or four days the irregularity disappears, the elevations become more or less conical, and blood vessels are very plainly visible near the surface of the newly organized tissue. The mature unruptured follicles are large, convex, smooth and semi-transparent.

The only difficulty in telling in advance how many eggs should be found in the tube arises from the fact that sometimes two ruptured follicles are very close to one another, and that occasionally two eggs may escape from one opening, either on account of the internal rupture of one follicle into another, or by the rupture of a follicle with more than one egg. In the case of animals killed prior to ovulation only the ovaries were removed, while in those killed subsequent to that process the Fallopian tubes, stripped of as much connective tissue as possible, were also preserved.

As a fixing agent of general utility Zenker's fluid gave satisfactory results. Gilson's fluid and strong Flemming were also employed. After dehydration of tissues with alcohol either cedar oil or xylol was used in preparation for embedding them in paraffin. Those which had been treated with the former seemed less brittle than those upon which the latter had been employed. All sections were cut 0.01 mm. thick and stained in lots of 25 slides in Delafield's or Harris's haematoxylin. For preliminary work this stain was entirely satisfactory, but Heidenhain's iron haematoxylin was substituted before the study of an important or interesting series was considered complete.

For demonstration of the fatty globules of the cat's egg strong Flemming's fluid was used, and it is a satisfactory reagent for the purpose. The fixation of the blackened fat is not permanent however, and to stand a very few minutes in a mixture of benzole and turpentine will completely remove it. Since Delafield's haematoxylin does not stain well when the tissue is preserved in Flemming's fluid, this fixing agent was used only sparingly and for the specific purpose of studying the fat globules or spindle fibres, which are perhaps a little better indicated through its use than through that of Zenker's fluid.

In the endeavor to relate to one another the different stages of degenerating eggs found abundantly in almost any ovary, or to distinguish between normal and abnormal eggs, Mallory's connective tissue stain was found to be of great service. Although its action is a little uncertain, that is to say, although in different ovaries the same element, the granulosa cells for example, is not always stained exactly the same color, upon a number of slides preserved in the same way and subjected together to the stain for the same length of time its action is accurate. It stains the follicular fluid of different follicles in a perfectly specific way, *i.e.*, the follicular fluid through several hundred sections of one follicle will be stained the identical shade of blue, while the stain of a neighboring follicle will be of a different shade, but will possess the same uniformity of coloration. This fact seems to be related as closely to the physical condition of the contents of the follicle as to any variation in their chemical composition corresponding to their stage in degeneration. But, whatever the cause, the result is the same. Different follicles are aligned in the same group, and may be compared with one another. The common characters of the group may thus be obtained, and the different groups arranged in the order in which their morphological characters show that they have departed from the normal.

The use of the stain is twofold. By the difference of its action it emphasizes the fact that different stages of degeneration exist contemporaneously, *i.e.*, that it recurs more or less periodically. Farther, by its ability to group the stages, it performs the equivalent of a reduction in the number which it is necessary to consider.

Living cat eggs were obtained both from the ovary and the Fallopian tubes. In the former case the ovary was placed under the binocular microscope in 0.7 per cent salt solution in a Syracuse dish. Pricked with a needle and subjected to a slight pressure, large follicles readily give up the eggs they contain, each surrounded by its discus proligerus. So surrounded, the egg, having once been located, is an object easily recognized by the naked eye, and by means of a fine pipette it may be readily transferred as desired.

After ovulation had occurred, eggs were obtained by taking a portion of the Fallopian tube, placing it with a few drops of salt solution on a slide on the stage of a dissecting microscope and with a dissecting needle or other instrument stroking it gently in the direction in which the eggs would normally move. Although R. Van der Stricht ('08) states that this method was suggested to him by M. Tourneux of Toulouse, it seems to have originated with William Cruikshank, 1797, and to have been commonly employed by Martin Barry in his researches in embryology.

Having been obtained, the eggs may be examined either with or without a cover glass. Some tube eggs showed many spermatozoa which had reached the zona, or were lying between the coronal cells surrounding the egg. The same condition is noted by O. Van der Stricht ('09) in the bat and by other observers in various animals.

Sexual season

The domestic cat may have from two to four sexual seasons during a single year, but only one if the animal become wild, (Heape '00). One of the chief periods of sexual activity occurs during the early spring, after the winter rest, and in the region of New Haven is at its height during the latter half of February and March. R. Van der Stricht ('08) records it as a fact that for animals in captivity the period of oestrus is delayed several weeks.

In the absence of the male oestrus will continue nine or ten days, (Hamilton '96). R. Van der Stricht ('08) states that it will last two or three days, presumably after the first copulation. Upon the different lengths of the period of heat in the absence of the male, or with the male present and allowed to inseminate the female, or present and not allowed to pair, no conclusive evidence is here offered, the maximum period of heat having been attained under none of these conditions. No cat studied has shown any unwillingness to be covered within fifty hours after the first pairing. Cats which had not been impregnated, but which had been approached by the male, gave heat reactions up to six full days, but the only animal in heat so long was inseminated at the end of that time. Since the repeated presentation of the male without the occurrence of copulation serves to keep the female longer than she would otherwise be in oestrus (Van Beneden),[1] no data obtained after this fashion would fall in the same category with those of Hamilton.

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[1] This statement was never published by Van Beneden himself, but with his consent it was made by Winiwarter and Saintmont. Archives de Biologie, T. 24, p. 129.

If kittens are taken away from a cat within a few hours of their birth, the mother may come in heat again from three to six weeks after parturition, a fact which is in substantial agreement with those noted by Winiwarter and Saintmont ('08).

Ovulation

Ancel and Bouin ('09) distinguish between mammals with spontaneous ovulation, and those in which it is provoked by copulation. In the first class they place the primates, including man, and in addition the dog, horse, cow and pig. All of these are probably properly classified; in fact any animal which can be artificially impregnated may be placed in this group. In the second class they include the rabbit, guinea pig, mouse and cat. With respect to the mouse, their classification is incorrect, Sobotta ('95) and Kirkham ('07) having proven that the ovulation of that animal is independent of pairing. Regarding the ovulation of the rabbit and guinea pig there has been difference of opinion among those who have investigated the matter. It would seem probable that the rabbit is correctly (Heape '05), and the guinea pig incorrectly placed in the second class (Rubaschkin '05). The ferret also belongs in the second group (Marshall '04).

In a preliminary note ('10) the writer has already been able to confirm the statement of Ancel and Bouin with respect to the ovulation of the cat, a statement previously made by Winiwarter and Saintmont ('08). The evidence will follow in detail, and is summarized in fig. 1.

Of a series of ten females killed at periods ranging from 23 to 50 hours after pairing, six had ovulated, and another, the first in the series, would certainly have done so within the longer time, if one may judge at all from the condition of the largest follicles in her ovaries. On the contrary, in a second series of five animals, *not allowed to pair*, none had ovulated within 50 hours, nor indeed within a much longer time, after being observed first to be willing to pair.

One animal in the second series, allowed to live one week after the close of an observed period of heat of six days, showed three

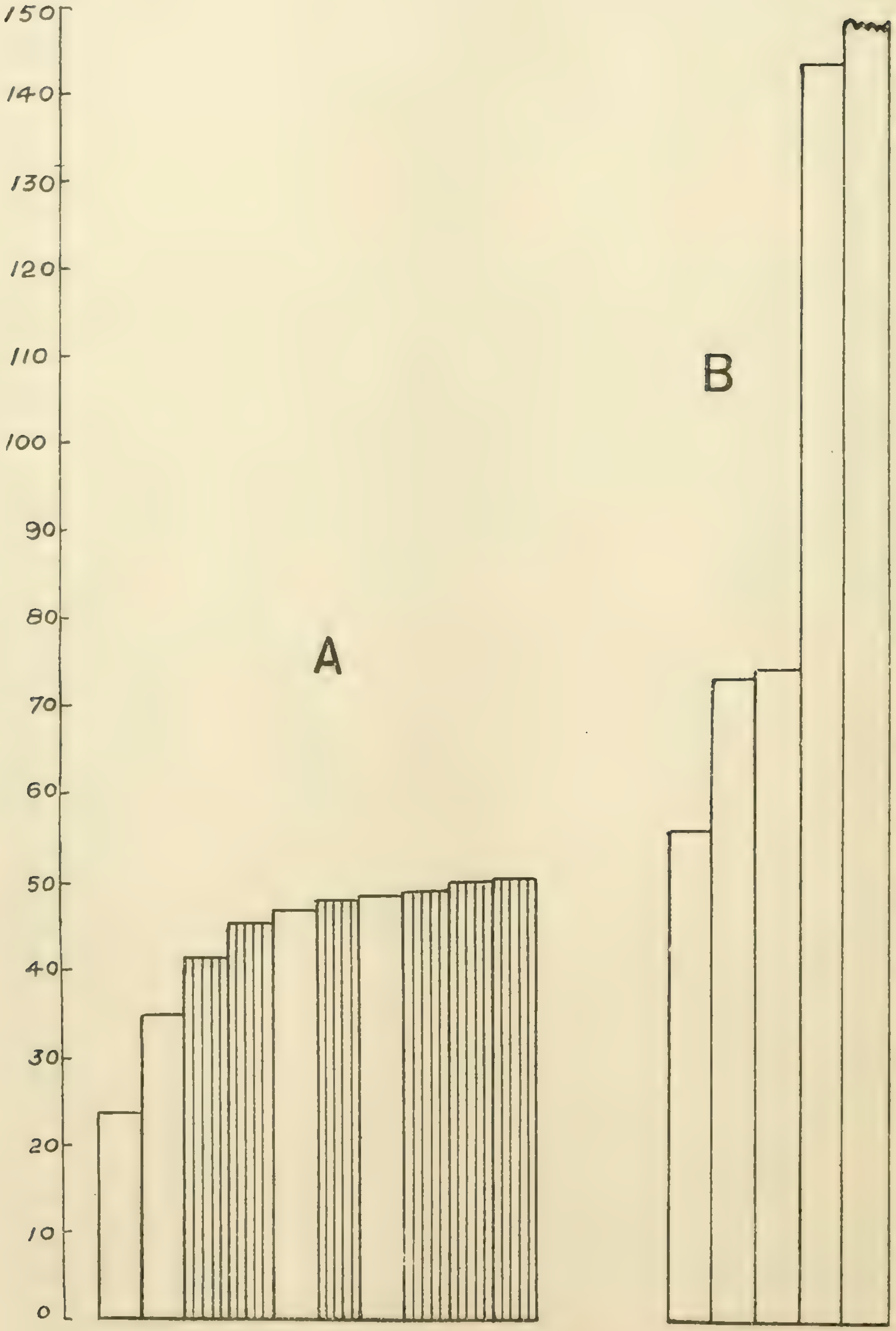

Fig. 1 Blocks represent individuals. Shading indicates occurrence of ovulation. *A*, animals all inseminated; *B*, none inseminated. Time: (*A*) hours elapsed after pairing. (*B*) after first willing to pair.

well marked series of degenerating eggs in its ovaries. This animal had really been intermittently in heat for not much less than a month before she was killed, so the hypothesis seems a very probable one that the eggs observed represent three distinct groups which had reached the limit of their independent ovarian evolution, and had degenerated after waiting in vain for the appropriate stimulus to their farther development.

From the facts presented it appears then that at least 70 per cent of a number of cats may ovulate within 50 hours after pairing, while, even in much longer periods, that process occurred in none of those not allowed to pair. In addition in the ovaries of animals not allowed to pair there may appear well individualized groups of eggs undergoing degeneration in consecutive order. It seems difficult therefore to avoid the conclusion that in general, if not always, in the cat ovulation is dependent upon pairing, although Bonnet ('97) twenty years ago, found a tube egg in an animal which he believed had been confined beyond the possibility of impregnation. It is interesting to note that the egg in question was accompanied by two corpuscles which Bonnet supposed to be the two parts of the divided first polar body. The writer finds the division of the first polar body not of very common occurrence, especially in normal eggs.

One of the most characteristic phenomena related with heat is the unusually abundant blood supply to the uterus and ovaries. Its importance in bringing about ovulation in the rabbit has been shown by Heape ('05) who demonstrated experimentally that the rupture of the follicles will not occur in the usual manner seven to ten hours after pairing, if the excessive blood supply to the ovaries be cut off. In the cat the blood supply seems independent of pairing, *i.e.*, the calibre of the ovarian blood vessels seems about the same during heat whether pairing does or does not occur. But since ovulation does not take place without pairing, it follows either that the estimate is incorrect that in the two cases the blood supply is the same or that there are other factors than blood supply which determine the occurrence of ovulation. Winiwarter and Saintmont ('09) describe a smooth muscle tissue in the theca externa and in the hilum of the ovary which they believe functions in coöperation with blood pressure to rupture the mature follicle.

Sobotta ('96) observes that in the mouse bleeding occurs ordinarily neither at nor after the rupture of the follicle. Clark ('96) working upon pig's ovaries, reports upon the contrary that more or less blood is found in all the recently ruptured follicles, and with his observations those made upon the cat by the writer more closely agree. Some follicles were nearly choked with blood, but there is a great variation in the amount present. The impression given is not that the rupture of the blood vessels of the theca interna causes the rupture of the follicle, but rather that it is dependent upon it. Since the rupture of the follicle and the loss of follicular fluid diminishes the pressure in the antrum, the blood pressure in the small vessels is an unbalanced force, which, in some cases more than in others, causes the blood to burst from its confines. In extreme cases it seems to gain direct access to the antrum of the follicle, but usually the blood spaces lie in elevated portions of the theca interna or are bounded by the membrana propria.

The usual process followed in ovulation is that an accumulation of fluid causes the distension of the follicle, which consequently bulges beyond the general surface of the ovary. The external follicular wall becomes very thin and finally its granulosa layer divides at the point nearest the periphery. The rupture of the tunica albuginea follows. With the yielding of the layer last mentioned either the elasticity of the theca, or its active muscular contraction or the pressure from the surrounding stroma decreases the size of the antrum. As a consequence part of the follicular fluid containing the egg, which at this period lies practically free surrounded only by its zona and corona (fig. 3) is forced from the follicle.

The membrana propria is not sufficiently elastic to adjust itself to the new dimensions of the follicle and is thrown up into folds, beneath which the connective tissue fibres and lutein cells follow more or less closely and form a supporting axis. The granulosa cells are thrown into a corresponding series of elevations. Upon preliminary examination they seem to be a very thick, loose layer, but they really are only a thin one, sometimes only two or three cells thick, and their apparent depth is only the approximate height of the folds into which the membrana propria is thrown. In other words, the section of the young corpus luteum shows with variable distinctness the sinuous outline of the membrana propria, following the curves of which, on the inner side, there is a thin layer of granulosa cells. Often large blood spaces occur in the apex of the folds of the membrana, or in the cores of connective tissue, upon which depends the more or less lobulated condition of the section of the corpus luteum next older. This condition is shown in fig. 2.

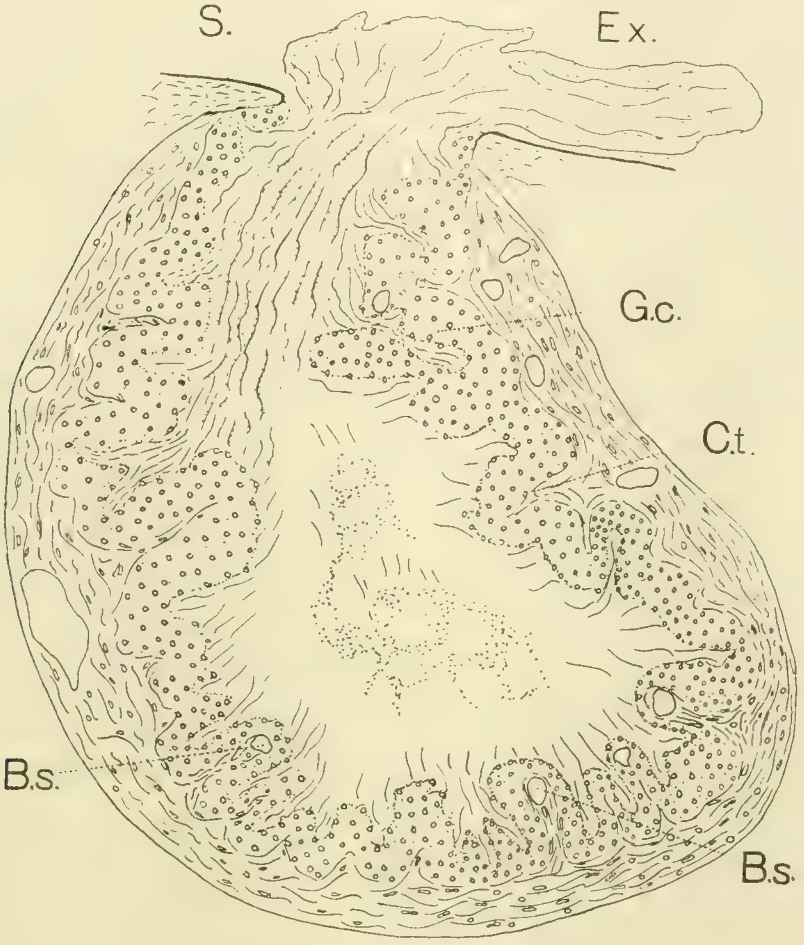

Fig. 2 Recently ruptured follicle, *i.e.*, young corpus luteum. Semi-diagrammatic. *B.s.*, blood space; *C.t.*, connective tissue elements of theca interna forming axis of fold; *Ex.*, coagulum of extruded liquor folliculi; *G.c.*, layer of granulosa cells; *S.*, free surface of the ovary. × 32.

Fig. 7 shows the section of an egg which was found escaping from the ovary. It will appear at once that in a number of details conditions in this case do not agree closely with the preceding general description. In this connection several facts should be noticed: (1) Of five other eggs discharged from the same ovary at this period four had been penetrated by spermatozoa. The other was not found. (2) Usually in the cat all eggs discharged at one ovulation are found in approximately the same stage of development. (3) In this case six eggs escaped from a single ovary, whereas the average number is about two, and four the largest number observed by the writer in any other case in the cat. (4) The recently ruptured follicles which had contained the other eggs conformed to the common type described in the last paragraph. A section of one of them provided the outline for fig. 2. (5) The follicle is a very small one having less than one-eighth the average volume of the other ruptured ones in the same ovary. (6) It is at the very extremity of the ovary. The foregoing data suggest that the rupture of this follicle may have been delayed on account of the unusually large number of the others and the fact that they stood nearer the hilum of the ovary, *i.e.*, in more direct relation to the blood supply. Since the ovary was removed from the body of the animal so soon after the follicle discharged its contents its difference in appearance from the others may be, and probably is, due to a considerable extent to lack of time to develop the characteristic appearance of the recently ruptured follicle in this animal.

Eggs in process of leaving the ovary have been figured by Sobotta ('95) for the mouse and O. Van der Stricht ('01) in the bat. Martin Barry's ('39) figure and description refer to a rabbit's egg forced from the ovary by artificial pressure.

Fig. 6 shows the same egg as fig. 7. It was studied and drawn under the binocular microscope after preservation but before being sectioned. The rosette shaped mass near the surface of which the egg is seen to lie is composed only of coagulated follicular fluid and extruded granulosa cells. In preservation the delicate texture and high degree of transparency of this matrix in which the egg lies was lost, being replaced by the characteristic opacity of coagulated albumen. The picture is somewhat of a curiosity since no other mammalian egg seems to have been observed under such conditions.

O. Van der Stricht ('08) agrees with Bischoff ('45) that in the ovary of the dog the development of the corpus luteum is begun before the rupture of the follicle. He finds that certain 'hineinspringende Zöttchen und Fältchen' seen by the earlier investigator in the mature follicles of the dog before ovulation are caused by a movement of the connective tissue elements of the theca interna, as a result of which the membrana propria does not retain its usual position, but is thrown up into folds, on which in some cases a series of secondary folds is superimposed. The effect of the folding of the basal membrane is to loosen the granulosa cells effectually and cause them to rise irregularly, giving the interior of the follicle the tufted appearance noted by Bischoff. With ovulation the change in the follicle is apparently one in degree only, and not one in kind.

In only one case in the cat was anything noted comparable to the above. In a single follicle which had already ruptured its granulosa layer, and put the bounding layer of the tunica albuginea under extra tension, the membrana propria of the deeper wall was thrown into the characteristic wrinkled form. Since the most that patient search has ever revealed in any other unruptured follicle is an occasional slight irregularity in the membrana, and since a follicle just ruptured (fig. 7), and with the egg still retained in the clotted fluid near the opening showed a considerable upheaval of the granulosa cells, we must conclude that in the cat the first step in the formation of the corpus luteum is contemporaneous with the rupture of the follicle.

In general, ovulation occurs only after the first polar body has been extruded, but there are occasional exceptions to this rule, two of which have come to the writer's notice. The first was manifestly a case in which an egg was escaping through a rupture in the cortex of the ovary caused primarily by the bursting of a follicle other than its own. It is of especial interest in this respect, that the egg whose discharge is the secondary phenomenon, is being passed out surrounded by its entire follicle, with the thecae intact. Incidentally, it might be observed that there are leucocytes present in its antrum, and that its follicular fluid has the appearance of a homogeneous coagulum. These are both indications that degeneration of the follicle and its contents is in active progress. In connection with the discharge of this complete follicle it is interesting to note that in various corpora lutea small follicles have been noted whose connection with the stroma has been severed. They lay as a result completely surrounded by the cells of the corpus luteum, and suggest the possibility that the condition above described is not unique.

The second case is not so clearly defined. In a certain ovary there were four ruptured follicles, and four tube eggs were found, three of which were in the two-cell stage, while the fourth possessed a germinal vesicle. In order to escape the conclusion that a follicle may occasionally become distended with follicular fluid and rupture in the usual way but in advance of the maturation of the egg, one must believe that in this case as well as in the last the egg in question was discharged as a result of a secondary condition, the disturbance of equilibrium within the ovary consequent upon the rupture of the first follicles to burst. Or possibly of five eggs which may have been discharged one was lost. The immature condition of the egg was indicated by the solid condition and the large mass of the discus proligerus which surrounded it. The condensation of the chromatin to form chromosomes had not begun. Curiously enough, a second smaller, more immature egg was embedded in the same fragment, a fact which may or may not be significant, but indicates at least that in a sense the follicle from which these eggs came was abnormal.

Winiwarter and Saintmont ('08) note in a cursory way that certain facts have suggested to them that there may be a regular alternation in the functioning of the ovaries. Since the average activity of right and left ovary is the same and there are many more cases of unequal than equal activity at any specified ovulation, it would seem at first sight as though there might be some foundation for the idea. But when one considers the cases in which the total number of eggs discharged at one ovulation is even, it appears that in one-third of the cases they are derived in equal numbers from each ovary, and that in half the remaining cases the right contributes more than the left, and *vice versa.* This is so near the result one should expect from a chance or arbitrary division of labor, since the average number of eggs discharged is not far from four, that anything but a very guarded statement of alternation in functioning seems rash.

Approaching the same subject from another point of view, the following table in which a comparison is instituted between the number of follicles recently ruptured and the number of corpora lutea corresponding to the last previous pregnancy shows that an unequal manifestation of activity at one ovulation is likely to be followed by a reversal in functioning at the next, but that there are other factors concerned, so that unequal activity may follow upon equal, or even that upon two successive occasions the same ovary may discharge the larger number of eggs.

CAT	RIGHT		LEFT		REMARKS
	Eggs discharged	Corpora lutea	Eggs discharged	Corpora lutea	
XXVII	6	1	1	3	Alternation
VII	2	2	4	2	Unequal following equal activity
XXXII	5	1	0	3	Alternation
XXV	3	3	1	2	Larger number of eggs from same ovary twice in succession

Maturation and pairing

The ovaries of half-grown kittens far from sexual maturity show many eggs undergoing maturation. Those of one such animal, for example, showed fourteen eggs with polar spindles, a considerably larger number than is usually found in an adult. This fact shows that maturation may be inaugurated without pairing. The ovaries of animals killed at the beginning of heat also ordinarily contain eggs either with the first polar spindle, or second polar spindle accompanied by the first polar body.

The question regarding the future of these eggs does not long await an answer when once a true conception of the normal egg is formulated. Such a conception is to be attained by the study of tube eggs before fertilization or during the earliest stages of that process. These are chosen, first, because, they are proven by their condition to be normal, for they are the eggs which produce the new generation, and second, because they must retain most completely the characters of the mature ovarian egg. For an example of such an egg see figures 11 and 12.

Such tube eggs are approximately spheres. Each possesses a zona pellucida of considerable and nearly uniform thickness, within which there are no leucocytes or granulosa cells. The corona surrounding each has its radiating structure developed to the extreme. Therefore when ovarian eggs are found possessing the characters indicated they are to be considered normal. The follicles containing such eggs, moreover, exhibit a common set of characters which are serviceable in defining the class of eggs which is approaching maturity in full possession of its capacities for development.

The normal follicle, (fig. 3), just before its rupture, is provided with a thin granulosa layer usually only two to four cells thick, except in the region of the cumulus. The egg in it contains a second polar spindle, and is accompanied by a first polar body. It lies practically free in the follicle, surrounded by its zona and corona. Nevertheless it is perfectly plain that the thickness of the granulosa cells in the preceding stage was not uniform, but that they were aggregated to form a prominent cumulus jutting well out into the antrum. There are always many layers of cells between the egg and the membrana propria, although there is evidence of lacunae between the cells of the cumulus.

The normal ovarian egg nearing maturity must then be approximately spherical, must have a thick zona with no foreign cells within it, must not have had its corona affected in such a way that its power of assuming the highly developed radiate structure is lost, and finally must be in a follicle with a high cumulus, with many layers of cells between it and the basement membrane.

With these facts in mind, it is impossible to consider those eggs normal which are found undergoing maturation in the ovaries of sexually immature animals, or in those of mature animals, at the beginning or a period of heat, or at any time during heat, if pairing be not allowed, for these eggs never possess all and frequently possess none of the characters enumerated in the last paragraph. That is to say, all normal eggs under such conditions contain resting nuclei and the maturation of the cat's egg in so far as it foreruns normal development, is dependent upon pairing.

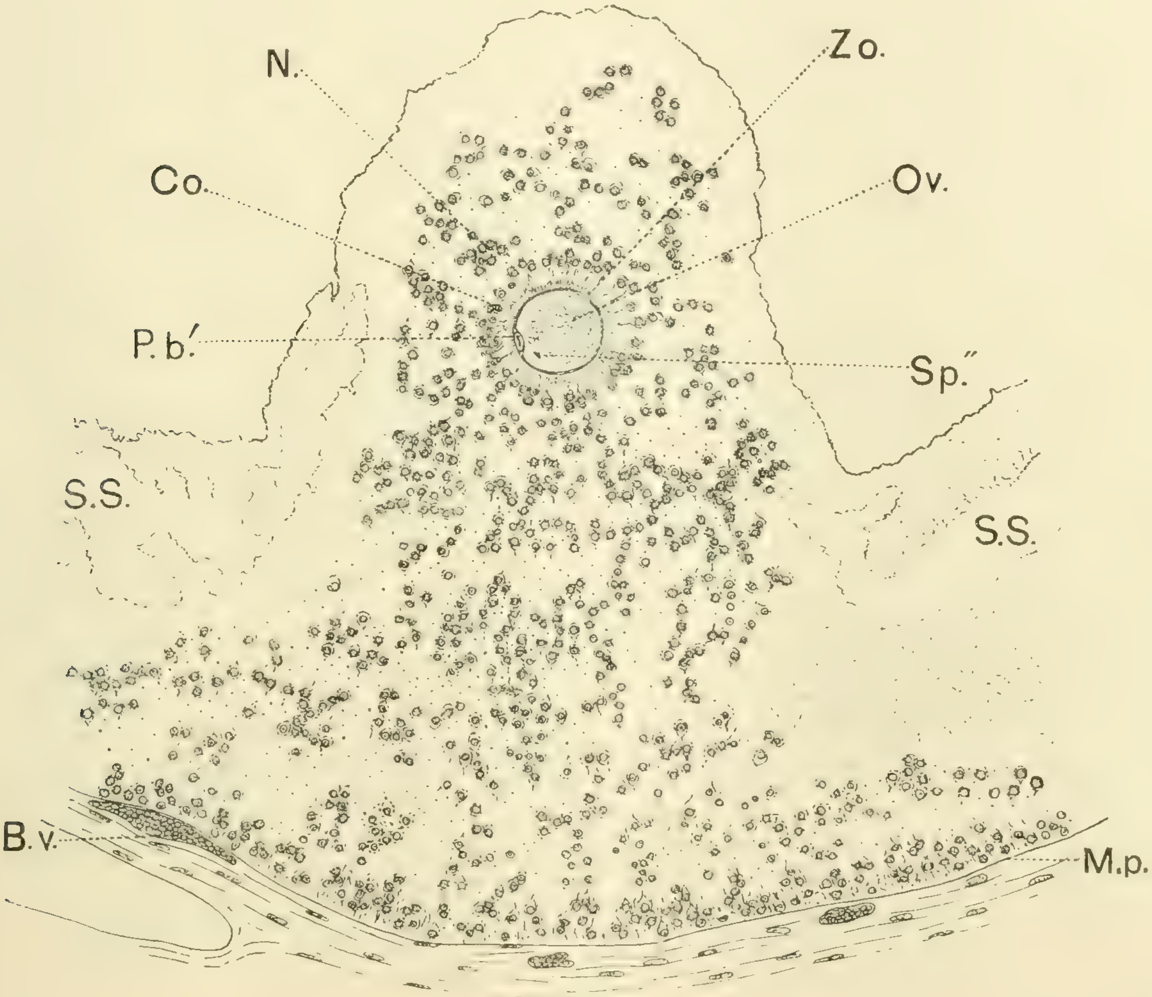

Fig. 3 Portion of a normal follicle just before rupture. *B.v.*, blood vessels; *Co.*, corona; *M.p.*, membrana propria; *N.*, nuclei of granulosa cells; *Ov.*, egg; *P.b.*, first polar body; *Sp''.*, second polar spindle; *S.S.*, shrinkage space; *Zo.*, zona. Note the round egg, perfect zona and corona, and great number of granulosa cells between the egg and the membrana propria. × 95.

The ovarian egg at the end of the period of growth

The living egg. The living cat's egg has a diameter ranging from 0.135–0.150 mm. and is surrounded by a zona 0.012–0.015 mm. in thickness. As compared with mouse and rat eggs, one of the most striking characteristics of the cat's egg is its opacity. This is largely due to the presence of an abundance of highly refractive nutritive globules, which are darkened by osmic acid. They vary from mere points to spheres of a diameter of 0.009 mm. but the most of them approximate a diameter of 0.006 mm. They tend to conceal the germinal vesicle, which is spherical, rather difficult to demonstrate, and has a diameter of 0.037 mm. The polarity of the egg is not well marked, *i.e.*, the greater part of the egg seems filled with the nutritive globules, but there is a small cup-shaped depression filled with granular protoplasm at that part of the periphery where the germinal vesicle lies. There is also a clear, superficial layer of like material about 0.015 mm. in thickness.

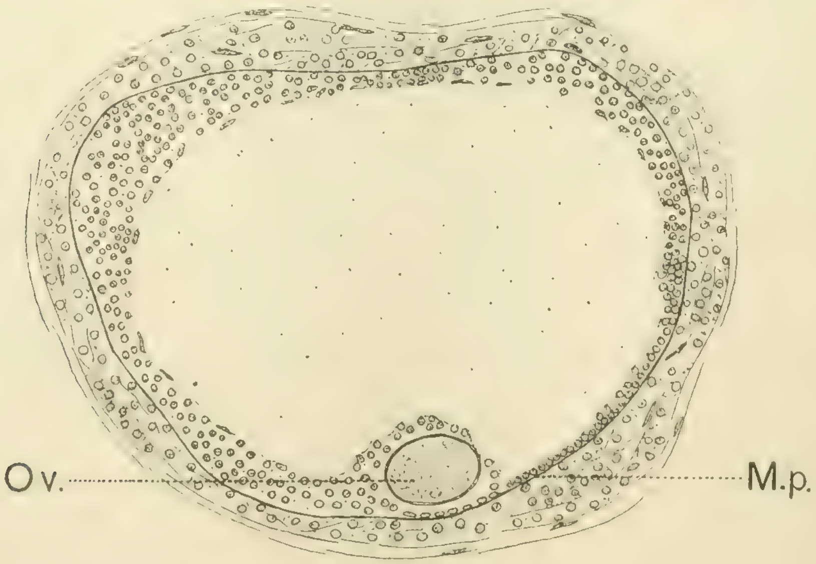

Fig. 4 Degenerating follicle. *Ov.*, egg; *M.p.*, membrana propria. Note the shape of the egg, the nearly complete absence of granulosa cells between the egg and the membrana propria, and the incomplete and morphologically imperfect zona and corona. × 95.

In fertilization stages the pronuclei are seen with difficulty or not at all.

The preserved egg. The section of an egg at the end of the period of growth shows a granular outer zone and an inner mass somewhat eccentrically placed, containing many vacuoles, from which the nutritive material has been dissolved by the treatment to which it has been subjected. In preserved material the germinal vesicle occupies a position at the periphery, entirely independent of the distribution of the yolk material within the egg. It may lie either in the clear protoplasm at the one pole of the egg, or at the opposite pole, where the nutritive globules are accumulated most abundantly, or at any intermediate point.

In germinal vesicles at this stage, one frequently finds the chromatin gathered together in a number of compact spheres of variable size, which on the whole are inclined to lie near the nuclear membrane. Holl ('93) has given a description of the nuclear changes during which these bodies appear in the mouse egg. He would have them originate in the nucleus from corpuscles of Schroen and migrate thence into the nuclear sap. He notes incidentally that the reticulum of the nucleus disappears entirely. Although this account is interesting, the condensation of the chromatin network in whole or in part seems sufficient to account for the origin of the spheres in question. These vary in number from about 35 to 10, the latter number apparently representing the more advanced stage of development and standing just before the organization of the spindle.

In preserved material, the contour of the nucleus in a mature egg is usually irregular, but the condition of the cytoplasm about it generally suggests that the lobulated or rugose state is due to shrinkage, *i.e.*, imperfect preservation. Van der Stricht ('09) considers an apparently similar phenomenon in the bat normal, and coupled with very late stages of the growth of the egg. It is to be noted that this form of the nucleus may be, and undoubtedly is, artificial and still indicate a special condition within, *i.e.*, very dilute nuclear sap, which is as fundamental a difference as would be a variation from its preceding spherical shape.

Eggs may attain to this stage and degenerate in the ovaries of sexually immature animals, or may do so after the formation of a first polar spindle, or with the first polar body and second maturation spindle. In sexually mature animals kept from pairing during a period of heat, degeneration may also intervene at any of the stages just indicated, but the actual nuclear changes appearing in this futile maturation are subject to a delay not manifested in eggs undergoing maturation normally in the ovaries of animals allowed to pair.

The first polar spindle

It has been noted already that the germinal vesicle in preserved material does not lie in any constant position with respect to the deutoplasmic accumulation within the egg. Similarly it is observed, as might be expected, that in the same kind of material the first polar spindle lies at either pole of the egg, or at any intermediate peripheral point. R. Van der Stricht ('08) has observed the same phenomenon.

This spindle seems to be organized from the contents of the nucleus at a variable period between twenty and fifty hours after pairing. It is found perpendicular to the surface of the egg (fig. 8) and there is no evidence that it occupies a preliminary paratangential position as in the mouse (Tafani) and guineapig (Rubaschkin). In this respect it agrees with the spindle of the bat's egg (Van der Stricht).

In thirty-three ovaries obtained from nineteen different animals, not one first polar spindle was found in an egg which appeared normal. This fact would seem to indicate that the first spindle discharges its function speedily. In this respect it is in sharp contrast with the second spindle which does not proceed to divide until the sperm head enters the egg. If it is true that the whole process of formation of the first polar spindle is consummated in a brief time, then it follows that the period during which a spindle might lie paratangentially must be still more brief and this fact may explain the failure to discover any spindles in that condition.

In first polar spindles in two eggs which would later have degenerated, it was possible to make an approximate count of the chromosomes. As the evidence of degeneration is not in the eggs themselves apart from a slight change in shape, the character of their chromatin content will be considered in detail.

The first of these spindles (figs. 5, *A* and *B* and fig. 8) was in the anaphase of division; its long axis was parallel to the plane of the section. The scattering of the chromosomes over a large area and the fact that all lay in one section simplified the matter of counting them. In this spindle where there is a minimum of coherence and of obscurity on account of one chromosome being superimposed upon another, twenty-four distinct pieces of chromatin may be seen. These bodies vary greatly in size and shape. The components of one pair are scarcely visible on account of their minuteness, while the largest ones are of a mass hundreds of times as great. Some are spherical, others are oblong or nearly square in optical section, and some are pear shaped, but none are filiform. Those of the last group seem on closer examination to be double, that is, to be composed of two chromosomes of unequal size adhering to one another. When this fact is taken into consideration in four cases (two pairs) and allowance made in addition for two pairs the existence of which beneath the others

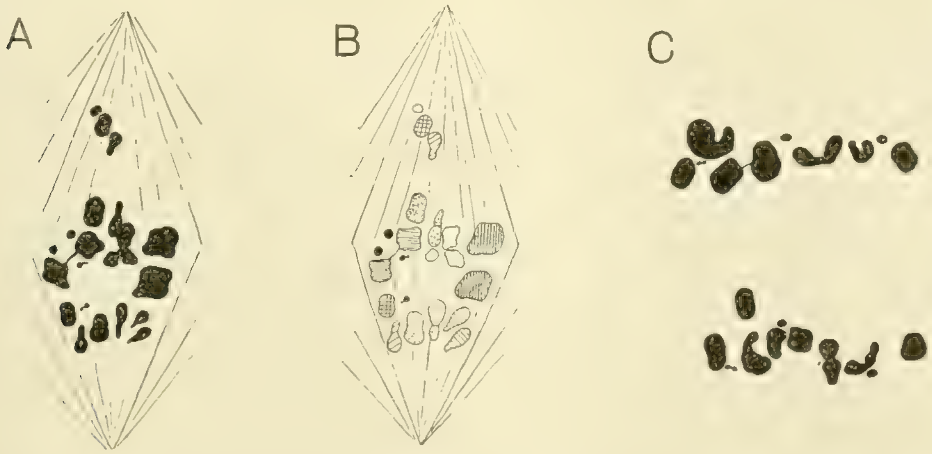

Fig. 5 *A*, first polar spindle in anaphase of division. *B*, same spindle showing one possible way of pairing the chromosomes. *C*, first polar spindle in telophase of division. The peripheral end of each spindle is upward.

cannot be definitely denied, the most that can be said with regard to number after the study of this spindle alone is that the individual tetrads are not less than twelve nor probably more than sixteen.

The second of the two spindles in question (fig. 5, *C*) is nearing the telophase of division and more crowding and overlapping of the chromosomes has occurred. Nine distinct masses of chromatin appear which were destined to have been eliminated in the first polar body. At the central pole of the spindle there are corresponding elements not quite as clearly individualized which would have furnished the chromatic portion of the second polar spindle. Farther study makes it clear that the first group includes certainly not less than twelve dyads. The second group strongly suggests that there are thirteen including a lagging heterochromosome.

The counting of the chromosomes in the last spindle described, therefore, greatly strengthens the hypothesis that twelve is the number of tetrads in the cat's egg. This idea must be modified, however, for in the first of the two there are eight bits of chromatin which are much smaller than the others, and in addition vary among themselves in size. That they are not fragments without significance appears from the definiteness with which they are paired. They represent eight individual dyads. Upon making a comparative study of these two spindles the one in the telophase of division shows at once two pairs of chromosomes which in size and shape correspond to the larger two pairs of the four of the first of the two spindles now being discussed. There is also a small, linear, chromatic element at each pole which from its size, shape and position it is perfectly justifiable to interpret as representing two very small chromosomes in apposition.

Thus the comparison of these two spindles gives some evidence in favor of an individuality of the chromosomes, although there seems no possibility of carrying very far the process of identification of individual chromosomes in different spindles. The point to be made in this connection is that fourteen becomes the minimum number of tetrads in the cat's egg. In fact, one might say that fourteen is probably the number of tetrads in the egg of this animal, if it were not that the uncertain evidence from the first of the two spindles above mentioned to the effect that there may be sixteen, receives some support from somewhat uncertain evidence of a similar nature from a second polar spindle studied, in which it seems possible that there may be sixteen dyads. Thus with respect to the reduced number of chromosomes in the cat's egg the most that can be said is that it is probably between fourteen and seventeen. Winiwarter and Saintmont believe that the somatic number of chromosomes in the cat is thirty-six and that the number in the first spindle is twelve (Arch. de Biol. T. 24, p. 197). They call attention to a similar discrepancy which they say occurs in the rabbit where the somatic number is forty-two and the reduced number ten or twelve.

The first polar body

Omitting those cases in which eggs escape from the ovary abnormally, as already noted, and speaking roughly with reference to the time element, all eggs which reach the tube organize the first polar spindle and extrude the first polar body within the ovary during the second day after pairing has occurred.

This polar body (figs. 9–10 and 11) does not disappear readily as the homologous structure does in the mouse (Kirkham '07; Lams and Doorme '07) but its chromatin is usually demonstrable even when an egg is far advanced in degeneration, or in the two-cell stage. Its chromatin may exist as a number of threads or granules, or may be gathered together in a single thread or in a compact mass. No evidence has been obtained indicating that the more compact forms ever take upon themselves the structure of a true nucleus, as noted by Kirkham ('07) to occur in the second polar body of the mouse, and incorrectly figured by Melissinos ('07) as occurring in both polar bodies of the same species.

In contrast to the condition commonly occurring in the mouse and noted by nearly all who have worked upon it, as well as in the bat (Van der Stricht '09) and the guinea pig (Rubaschkin '05), no polar bodies have been observed containing a mitotic figure. Very few have been found which by their equal division or equal distribution of the chromatin in the two daughter cells suggested that a mitotic division had really occurred. The first polar body as found with normal eggs usually shows a regular, more or less elliptical outline, but it may present a lobed or fragmented appearance, possibly artificial, or indicating the initiation of regressive changes.

The second polar spindle

The chromatic portion of the second polar spindle is very speedily organized from the residuum of chromatin after the expulsion of the first polar body. Like the germinal vesicle and first spindle it may lie, in sections of preserved material, at either pole of the egg or at any intermediate peripheral position. In the preparations studied by the writer it has always appeared perpendicular to the surface of the egg (figs. 9–10). As a rule it appears near the first polar body, *i.e.*, the latter suffers little displacement.

The achromatic portion of the second polar spindle is often very much reduced, and the combination of this feature with the arrangement of the chromosomes in practically one plane is characteristic of many spindles of this order. However, apart from the presence of the first polar body the best criterion serving to distinguish the spindles is the condition of the chromatin. In this connection there are at least three points to be noted. First, the difference in mass between two dyads is only half that which exists between the tetrads from which they are derived. Therefore the chromosomes of the second will vary less among themselves in size than those of the first spindle. Second, the first spindle soon proceeds to division. Its tetrads become divided and the chromosomes may be scattered throughout the greater part of the length of the spindle, corresponding dyads being seen in the two halves. Since the dyads of the second spindle do not divide until the sperm head enters the egg, this relation cannot occur in a second polar spindle in an ovarian egg. Third, a dyad usually shows some indication of being composed of two reniform bodies with their concave faces approximated. A narrow light area frequently appears between them.

In the second polar spindle the chromosomes are frequently adherent, or one might almost say confluent, so close is the relationship established. In such cases one finds a varicose thread plainly consisting of several elements and a number of detached bodies of chromatin which may consist of one dyad or of two more or less closely united. Under such conditions manifestly the number of distinct masses of chromatin is not the number of chromosomes except in the restricted sense implied by the derivation of the word. The greatest number of units which one can certainly decipher from the confused mass is the least which can be considered as the true number of chromatic elements in the spindle, provided always that eggs do not differ in respect to the presence or absence of accessory chromosomes.

Twelve dyads is a number frequently observed, but from the study of the first spindle it is plain that there are two very small ones and these two have not been seen in the second spindle, apparently being obscured by being associated with other larger ones. Except in one spindle the maximum number observed did not exceed fourteen. In the exceptional case there seemed to be sixteen. The reduced number of chromosomes is therefore probably not less than fourteen.

Fertilization

The unfertilized tube egg is surrounded by a corona in which the radiate structure is accentuated. The zona is thick. The outlines of the coronal cells gradually grow indistinct, while the nuclei still retain their staining power (figs. 11–12). The egg is spherical, and the deutoplasm is in the condition described for the ovarian egg.

The second polar body is formed after the sperm head enters the egg.

With early fertilization stages, sperm heads are found among the coronal cells in small numbers, a fact which is very reasonable, since naturally one of the first to arrive will fertilize the egg. With later stages they may be present in abundance. More than four hundred, almost all of which were distant from the egg less than its diameter, were counted in the immediate neighborhood of one two-cell stage. Some were found embedded in masses of granulosa cells which had become detached, and others on the epithelial surface of the tube at the points nearest the egg. If experiments with invertebrate eggs had not failed to show that the egg is able to exert a chemotactic influence upon the sperm, one would readily believe that confirmatory evidence of chemotaxis appeared here.

The second polar body

A second polar body is never found with an ovarian egg. Whenever with such an egg two corpuscles occur, which by their appearance suggest the possibility that they may be first and second polar body, they are invariably associated with a second polar spindle rather than with a female pronucleus.

The second polar body is in evidence before the sperm head has penetrated far into the egg (figs. 11–12). In early fertilization stages, fibres may be found passing from the chromatin of this body to the point at which it was constricted from the egg. The second polar body does not seem to have as dense cytoplasm as the first, nor to hold its definite outline as long. In some cases its chromatin is in several granules, in others in only one. Frequently it is possible to tell the two polar bodies apart by the different amount of chromatin which they contain, but when the first polar body's chromosomes have undergone a condensation and those of the second are less compact this criterion fails. Sometimes the position of the polar bodies with reference to the female pronucleus, or other evidence outside of the polar bodies themselves, may give proof of their nature, but in addition to all these cases, there are others where no clue can be found for the classification of the two cells in question.

Pronuclei

All of the small number of fertilization stages studied after having been preserved were in about the same condition. Both pronuclei were compact and very deeply stained (fig. 12). At the next stage, fusion had taken place in the center of the egg in a region of clear protoplasm, the deutoplasmic globules having moved toward the periphery. The chromosomes only are discernible, no evidence being noted of the presence of spindle fibres. The chromosomes differ from those of either of the polar spindles, being filiform in shape, with enlarged extremities.

Cleavage

In the two-celled stage there are usually found two peculiar bodies which stain dark blue with Delafield's haematoxylin and brown with Heidenhain's iron haematoxylin. They are variable in size, shape and location, are irregular in outline, and of a more or less granular texture. Very similar smaller bodies in number up to a dozen or more are sometimes found in ovarian eggs near the end of the period of growth.

Two-celled stages are surrounded by a zona which is of variable thickness. Sometimes it shows only at special points such as those where it covers the polar bodies, or where the egg outline is indented, as at the margin of the cleavage plane. At other times it is well in evidence (fig. 13) and shows as clearly as in mouse eggs for example.

In conclusion, the writer welcomes this opportunity of expressing his obligation to Professor W. R. Coe for advice and encouragement which have been freely given and for the inspiration originating in his rigorous but kindly criticism.

SUMMARY

1. Maturation stages are found in greater numbers in the ovaries of sexually immature than in those of mature animals.

2. Although eggs containing first or second polar spindles are found in the ovaries of sexually immature animals or in those of mature animals before copulation has occurred, maturation in all eggs which are destined to undergo a normal extra-ovarian development is dependent upon pairing.

3. The best criterion for distinguishing the first and second polar spindles, apart from the presence of the first polar body, is the condition of the chromatin.

4. The reduced number of chromosomes in the cat's egg is not less than fourteen.

5. In *preserved material* the germinal vesicle of the full grown egg and the first and second polar spindles agree in being found at the periphery of the egg at either the protoplasmic or deutoplasmic pole or at any intermediate point.

6. Two polar bodies are formed in all cases, the first in the ovary and the second in the Fallopian tube after the entrance of the sperm head into the egg.

7. The first and second polar bodies can usually but not always be distinguished.

8. Like the maturation of the egg, ovulation is dependent upon copulation and occurs about the end of the second day after pairing.

9. The average number of eggs discharged at one ovulation is approximately four.

10. There is no regular alternation in the functioning of the ovaries.

11. If the young are removed within a day of birth the mother may pass through a second period of heat and be impregnated within three to four weeks.

12. Very large numbers of spermatozoa may reach the Fallopian tube. There is a strongly marked tendency on their part to collect in the immediate neighborhood of the egg.

13. Mallory's connective tissue stain is of approved utility in the study of degenerating eggs and follicles.

14. Ovarian eggs may degenerate at any period of their development up to and including that in which they possess a first polar body and second polar spindle.

SHEFFIELD SCIENTIFIC SCHOOL OF YALE UNIVERSITY
January, 1911

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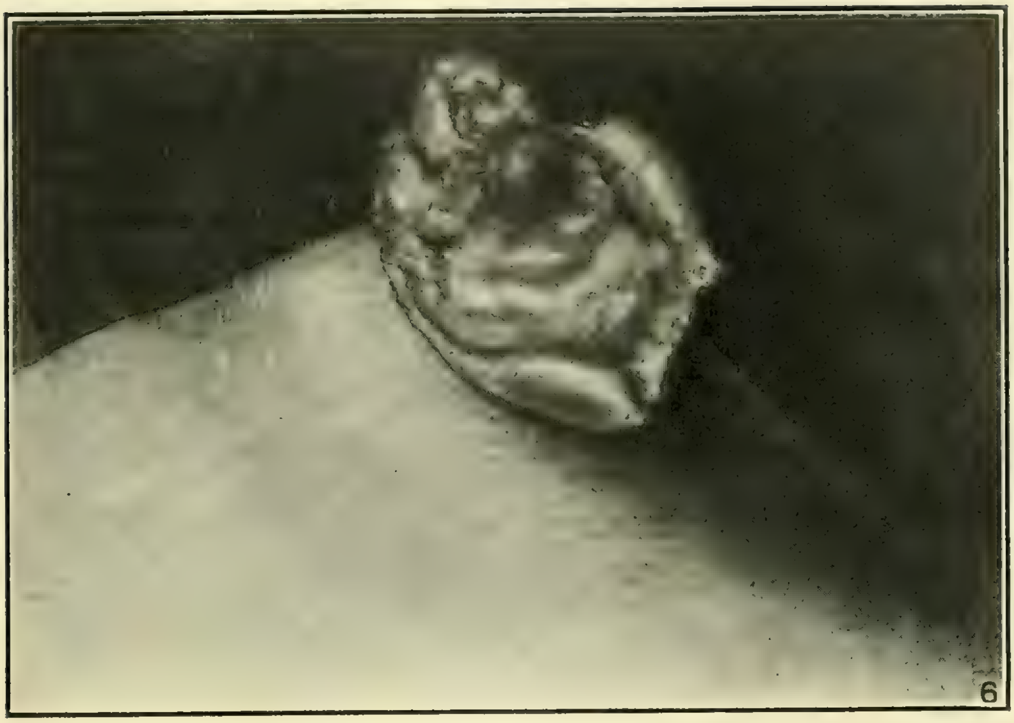
PLATE 1

EXPLANATION OF FIGURES

Fig. 6 An egg escaping from the ovary and drawn as seen under the binocular microscope before sectioning. The conspicuous folds are on the surface of a coagulated mass of follicular fluid containing granulosa cells expelled from the ruptured follicle. They do not in any way represent the torn edges of the cortex of the ovary.

Fig. 7 A section of the same egg. It is unfertilized and contains a second polar spindle accompanied by a first polar body. × 100.



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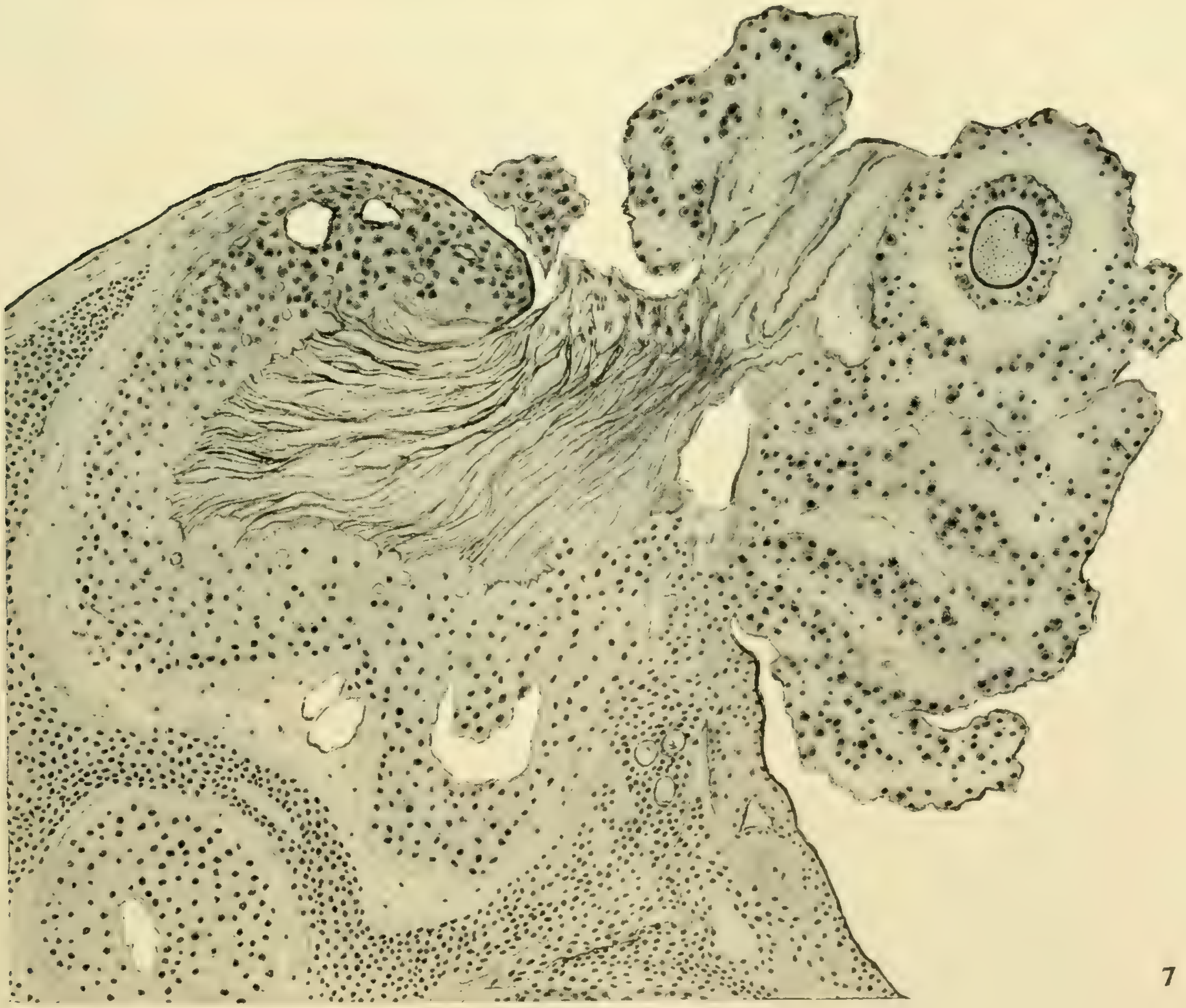

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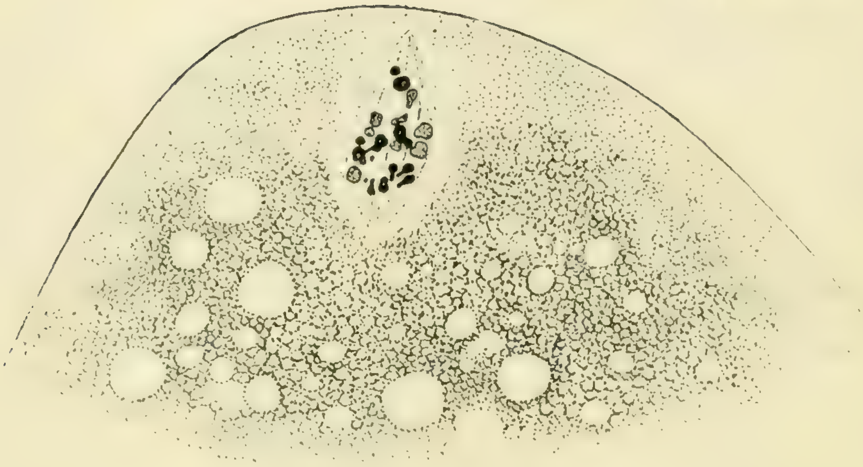

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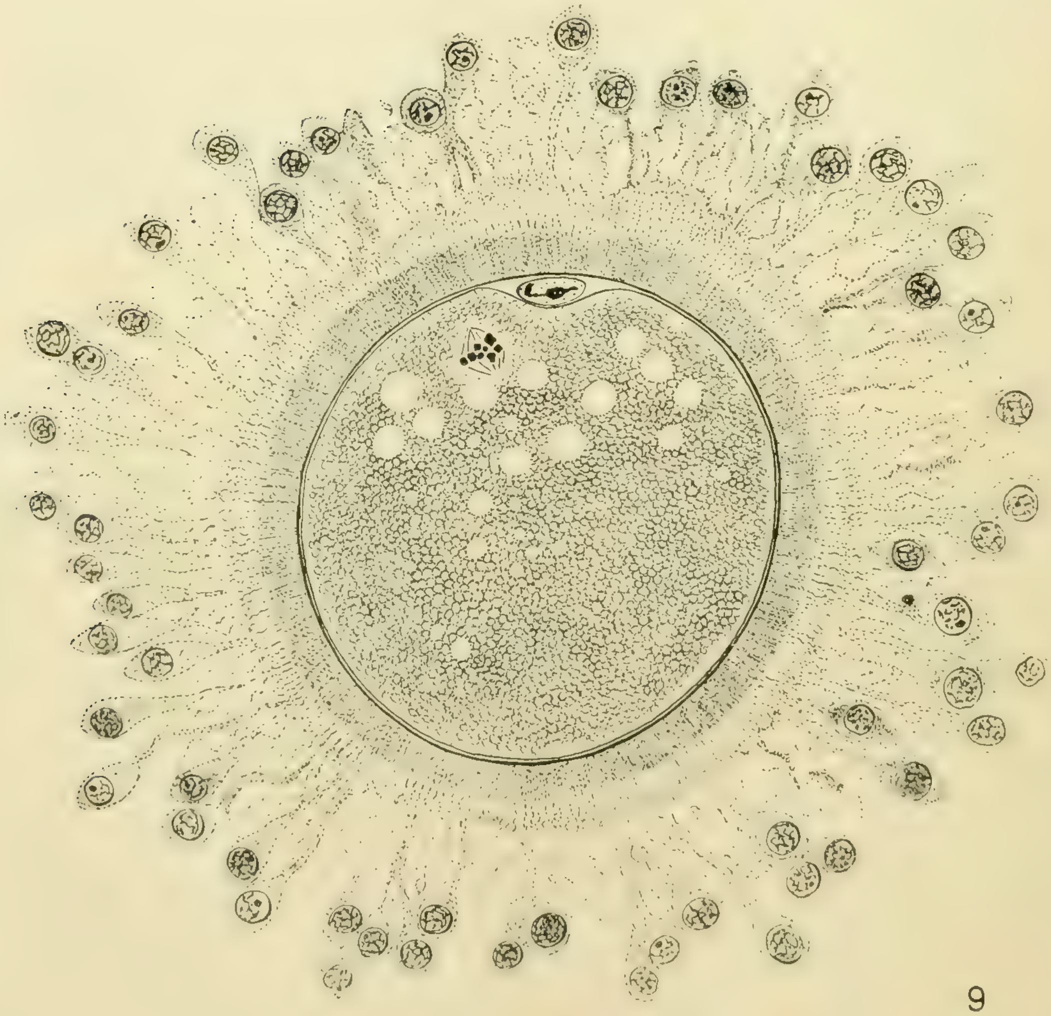

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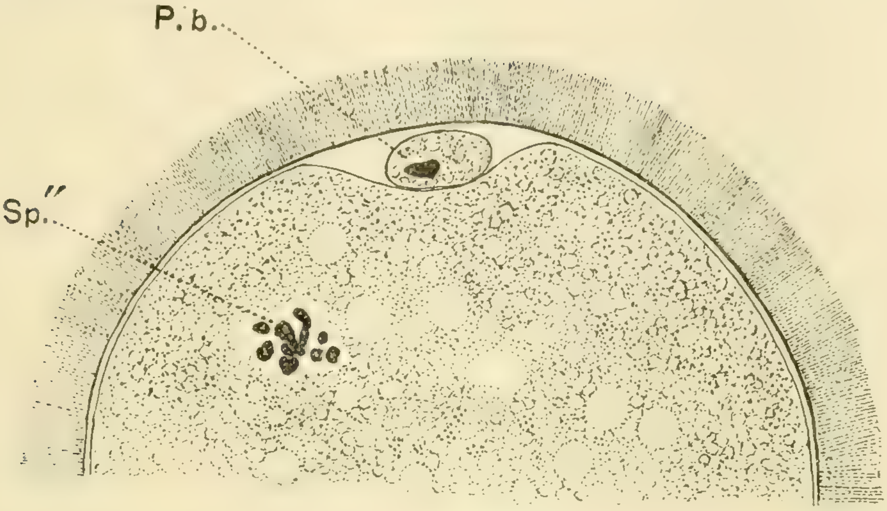
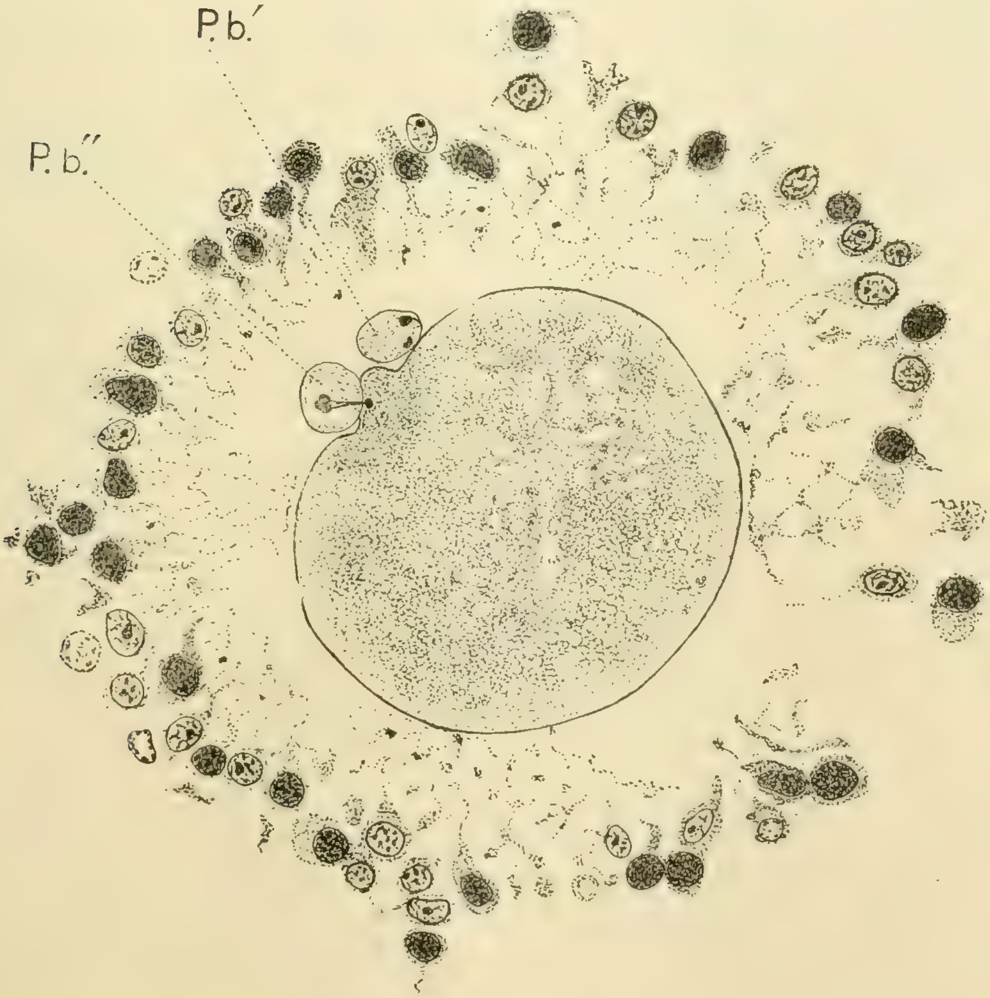
EXPLANATION OF FIGURES

Fig. 8 Ovarian egg showing the first polar spindle. From the condition of the follicle in which it was found it is plain that this egg was destined to degeneration rather than discharge from the ovary, fertilization and development into a new organism. For same spindle see fig. 5. A–B. × 950.

Fig. 9 Normal ovarian egg just before ovulation. It contains the second polar spindle and is accompanied by the first polar body. Note its circular section, thick zona pellucida and the radiating cells of the corona. For same egg see fig. 3. × 625.

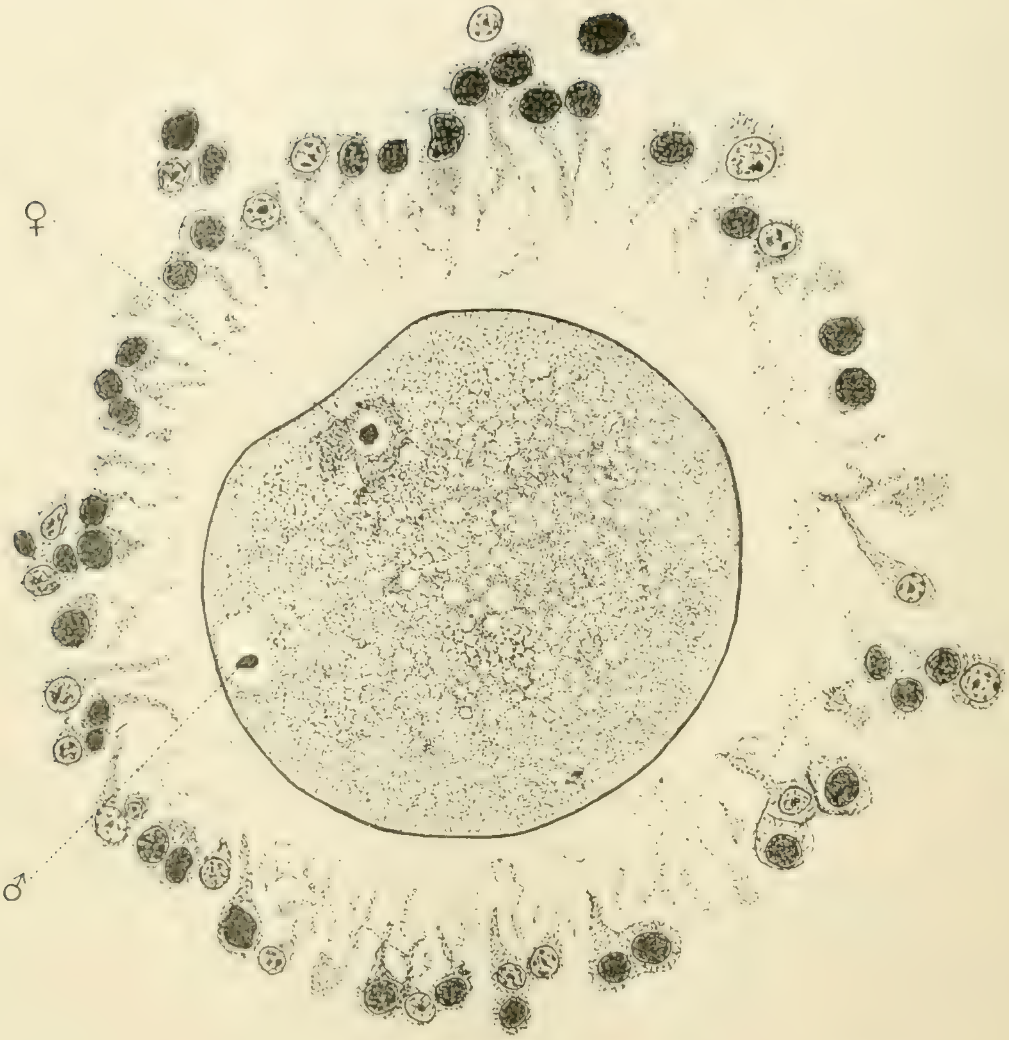


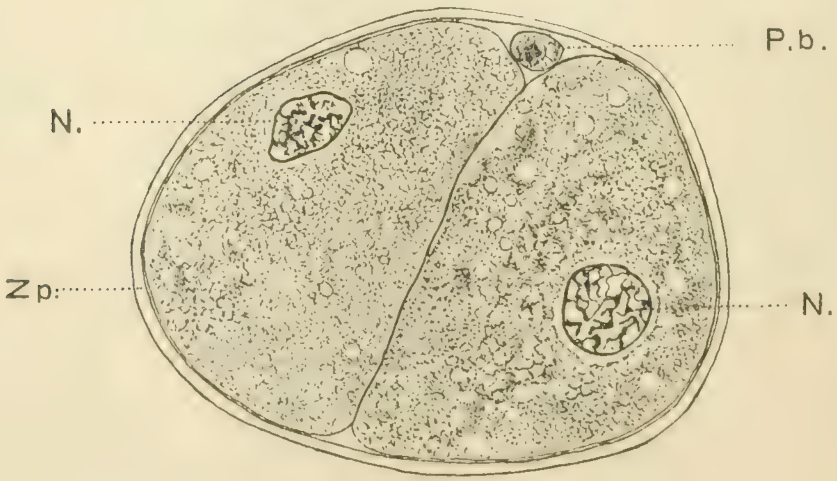


EXPLANATION OF FIGURES

Fig. 10 Polar view of second polar spindle in ovarian egg. × 790.

Fig. 11 A reconstruction of two adjacent sections of a fertilized tube egg. The second polar body has just been formed. Note that the arrangement of the nuclei of the epithelial cells accompanying the egg indicates the disintegration of a well developed corona radiata. The zona pellucida is not visible in this preparation. × 625.





EXPLANATION OF FIGURES

Fig. 12 A reconstruction of two other adjacent sections of the same egg shown in the preceding figure. The vacuole about the sperm head is probably an artifact produced by shrinkage due to an unusually thin cytoplasm surrounding it. The zona does not appear. × 625.

Fig. 13 Two cell stage accompanied by a polar body. Note the presence of a conspicuous zona pellucida. × 750.

THE EARLY DEVELOPMENT OF SPELERPES BILINEATUS (GREEN)

HUBERT DANA GOODALE

ONE PLATE AND SEVENTY-SEVEN TEXT FIGURES[1]

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[1] Descriptions of figures are given in the text.

INTRODUCTION

Early in May, 1904, while searching for the eggs of Plethodon cinereus, then unknown to me, I found a single batch of white eggs in early cleavage stages attached to the under side of a stone taken from a brook. Later, when the eggs were found in great abundance, they were identified as those of Spelerpes bilineatus. Search of the literature showed that only the later stages had been described in a brief paper by Wilder in 1899. According to Wilder, the eggs were not well known, having been recorded by Verrill only.

I was encouraged by Professor Charles L. Edwards of Trinity College to undertake the study of the development of these eggs. I desire to express my appreciation of this encouragement and to thank him for the use of the Biological Laboratory during the spring of 1905. Since the fall of 1904, the work has been carried on at Columbia University under the direction of Professor T. H. Morgan, to whom my best thanks are due for much kindly advice and criticism.

The experimental part of the work was undertaken by means of an entirely new method. It has proved possible to produce artificial spots of color in the living egg and thus follow out the shiftings of material in the normal egg, which hitherto has been done only by puncturing the egg. The results entirely confirm those obtained by the latter method, thus removing the objections of those who, like Ikeda, believe that results obtained from injured eggs are inapplicable to the normal egg.

PART 1. DESCRIPTIVE

The egg and its envelopes

The diameter of the egg varies from 2.2 to 3 mm. The upper hemisphere of the egg is snowy white but the lower has a faint yellowish tone. It is enclosed in several envelopes, shown in optical section in fig. 1. The delicate outer layer, *a*, is thin and difficult to make out except at the point of attachment, *a.*′ The inner surface, *c*, of the next layer, *b*, is tougher than the remainder since the latter is easily removed. The perivitelline space, *d*, filled with an albumenous fluid, surrounds the egg, *e*, to which is closely applied a very thin delicate vitelline membrane,[2] seen in the living egg only when the embryo begins to appear.

Occasionally two eggs are enclosed in a common membrane. One case was noted in which the outer gelatinous layer enclosed three eggs, two of which were closely packed into a common perivitelline space, while the third had its own.

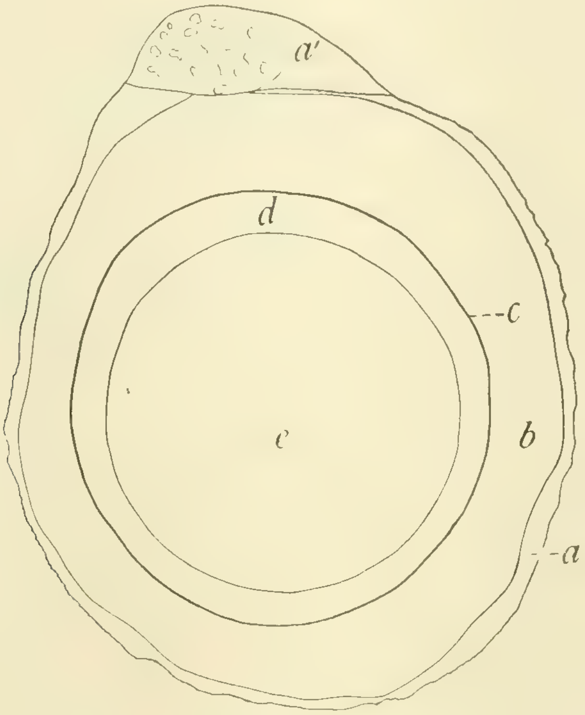

Fig. 1[3]

The liquid in the perivitelline space is under considerable pressure, for when the membranes are punctured they collapse suddenly, while the fluid squirts out with considerable force. If the egg be shelled from its membranes into a dish of water, it continues to develop for a time. The egg is very soft and flattens somewhat from its own weight. The first cleavage furrow in such an egg has been seen to flatten out into a broad trough, instead of the narrow furrow described below. Eggs, when removed from their membranes, if kept in sufficiently cold water may reach the gastrula stage, but before an embryo has appeared, the anterior wall of the gastrula has invariably broken out into a vesicle, resulting in the stoppage of development. This indicates, perhaps, a good deal of pressure by the invaginating macromeres upon the anterior wall. After the neural groove begins to appear, the embryo may be removed from the membranes without much, if any injury.

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[2] Van Bambeke ('80) calls this membrane the chorion.

[3] Descriptions of figures are given in the text.

The egg before cleavage, with especial reference to the yolk granules

A few hours after the egg is deposited, a number of small pits (from 2 to 6) may be seen scattered over the upper hemisphere of the egg. They may represent the entrance points of the spermatozoa, since physiological polyspermy has been observed in Urodeles by Jordan ('93), Braus ('95), Grönroos ('98) and others. Later on, they all disappear but one. Whether this one is an original pit, or has arisen meanwhile, I do not know. At any rate, the one remaining persists until after the earlier cleavages. It has been noticed by Jordan ('93) in Diemyctylus and by Eycleshymer ('95) in Amblystoma. It doubtless corresponds to the fovea of Schultze. According to these authors, it contains the polar bodies, but thus far I have been unable to find them, either in the living egg or in sections.

Preserved material shows a well-defined cap of lighter color than the rest of the egg. This cap, which represents the more protoplasmic portions of the egg, is not cut by the first cleavage plane in any definite direction. Vertical sections of such an egg, fig. 2*a* (drawn somewhat diagrammatically), show a well-defined arrangement of the yolk material into zones. Corresponding to the external light cap is a rather thin upper zone of finely granular material which extends well towards the equator, but does not reach it. It decreases in thickness towards its edges. Beneath this is a zone of coarser yolk material. The rest of the egg is composed of still larger yolk granules which often have a characteristic longitudinal, median groove (fig. 2, *e*). These zones are not sharply separated from one another nor are the yolk granules characteristic of one zone found exclusively in that zone.

The relative numbers of the various sized granules are, however, characteristic for a given zone. Fig. 2, *b, c, d, e* show the general size relationships between the granules of the three zones. Very large granules do not occur in the upper zone, nor do grooved ones appear in the middle zone. Likewise, the smallest granules do not appear in the lower zone.

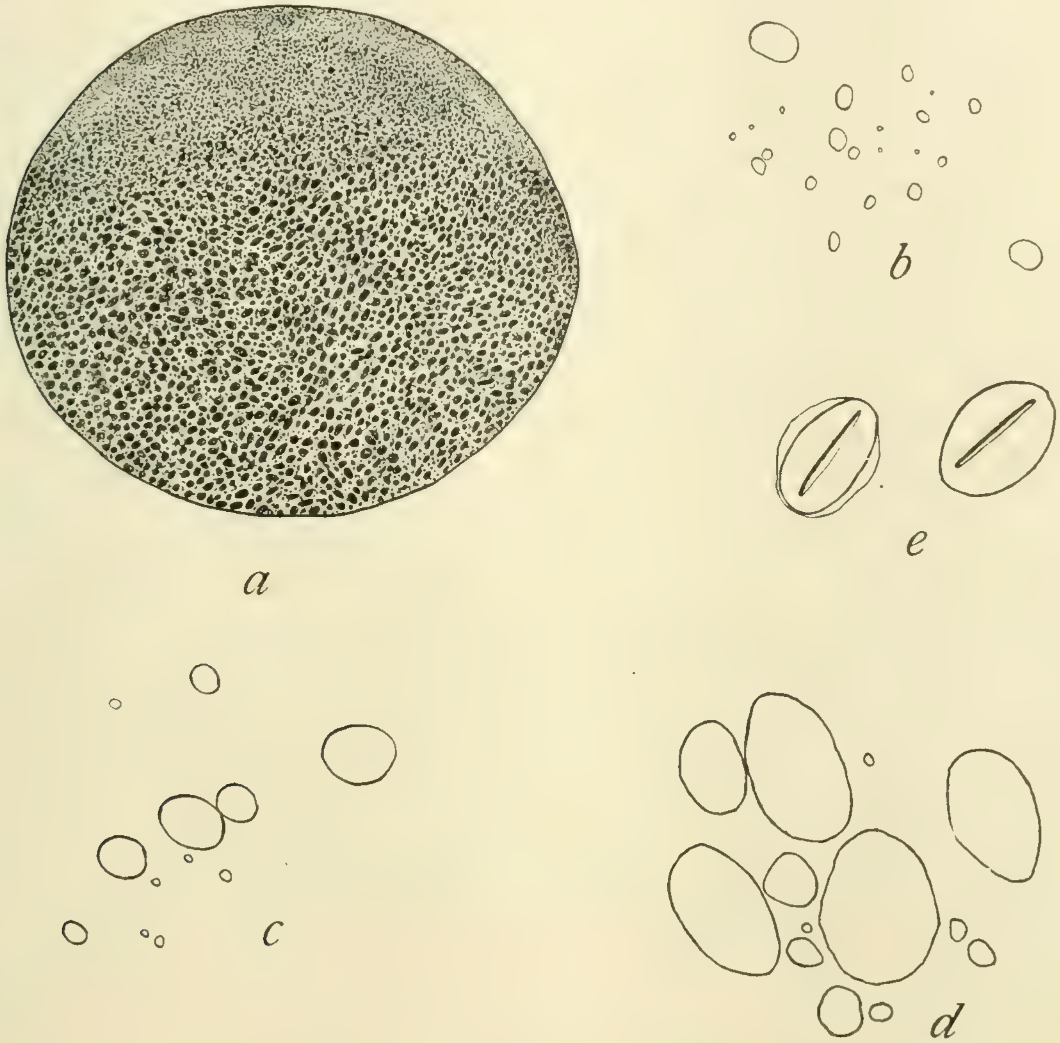

Fig. 2

Contrary to Jordan ('93), Houssay ('90) and others who maintain that the size of the yolk granules are of no value for determining the future history of the various regions of the egg, I have found that until after gastrulation at any rate, the differences in size of the yolk granules afford a highly efficient means for determining the distribution of the egg material. The yolk granules undergo little change during the early part of development.

A brief account of the later history of the granules is given here in order to afford a connected account of the distribution of egg materials into some of the embryonic organs. In fig. 14, a vertical section of an eight-celled stage, the general distribution of yolk granules has been represented; *f* indicates the region occupied mostly by fine granules, *i* the region of intermediate granules and *y* the region of large granules. The granules of intermediate size are being moved or are moving towards the outer equatorial regions of the egg. The same process at a later stage of cleavage is shown in fig. 16. Some large cells with large yolk granules are present in the center of the egg formerly occupied by granules of intermediate size.

By the time a well-developed blastocoele has formed the granules have become distributed as follows. In the roof are cells whose granules for the most part were derived from the upper zone, although some cells containing larger granules occur. At the sides of the blastula where the roof thickens, most of the cells have granules from the intermediate zone. The granules in the floor cells of the blastocoele are large ones which have maintained their original position.

At the close of gastrulation, when the neural plate has become distinct, we find that the upper zone of granules has maintained its position and now occupies the cells of the neural plate, except in its posterior part, which, together with the posterior part of the archenteric roof, the ectoderm exclusive of the neural plate and a small area immediately anterior to the last, have granules from the intermediate zone. Possibly parts of the mesoderm have a similar origin. From the lower zone is derived most of the roof of the archenteron including the mesoderm and notochord, besides the large ventral mass of yolk cells.

Even at a comparatively late stage, *i. e.*, when the somites have begun to develop, the source of the granules of some parts of the embryo can still be determined. For example all of the outer layer of cells have granules of intermediate size.

Cleavage

Since the external aspect of cleavage differs only in minor details from the usual urodelian form of total type, a very brief account will suffice.

Regular. It begins from twelve to twenty-four hours after the egg is deposited, three to five eggs of a clutch beginning at about the same time, while the rest follow in similar groups throughout a day.

The first indication of cleavage is a slight flattening at the upper pole, which soon becomes a deep broad furrow (fig. 1, plate 1). It soon closes, however, except at its ends (fig. 2, plate 1). In only one set was any thing similar to the "Faltenkranzen" of the frog seen. These were very small furrows, varying from one to eight, extending a short distance in various directions from the main furrow.

Some three hours after the first, the second furrow starts in like manner. The former becomes more distinct, due to a separation of its edges. This opening and closing of furrows may be observed throughout cleavage. The blastomeres soon shift, giving rise to the usual cross furrow (fig. 4, plate 1). Fig. 5, plate 1, shows one type of the third set of furrows.

The furrows of the fourth set, as a rule, are the last for a long time to extend below the equator. They do not even reach the lower pole, but usually join the earlier furrows less than 45° below the equator. Thus cleavage is confined to the upper hemisphere of the egg quite like that of Desmognathus fusca. (Hilton, '07). Plate 1, fig. 6, shows the upper hemisphere of an egg after the fifth set of furrows had formed. A later stage is shown in fig. 3, *a*, *b*, *c*, upper, lower, and lateral views, respectively.[4] There are 50 blastomeres visible from above and only 8 from below. In fig. 4, 130 cells are visible from above and only 7 from beneath. Another egg has 400 and 14, respectively. After the time represented by the last, there is a constant increase in the number of lower blastomeres. However, a few eggs were found in which cleavage from the start proceeded with considerable regularity in the lower hemisphere. Still other eggs are intermediate in their behavior. Thus the eggs of Spelerpes exhibit many of the amphibian types of cleavage.

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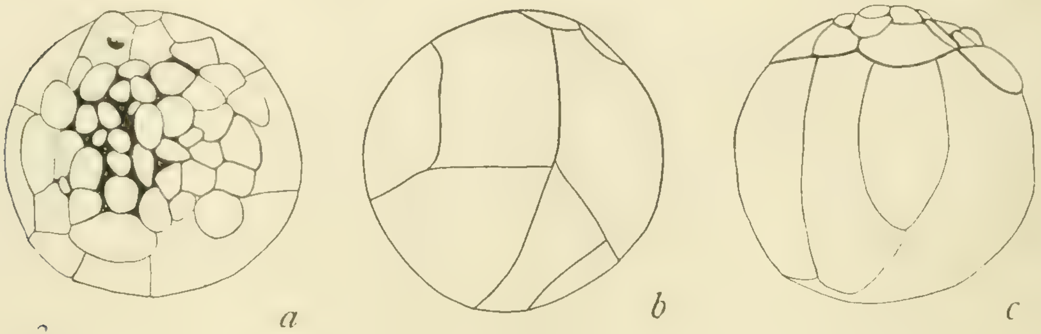
[4] The cavities represented between the cells are somewhat exaggerated, due to Merkel's fluid.

Irregular cleavages. Fig. 6 shows an extreme case in which the first furrow formed a small circle on the upper hemisphere of the egg which nevertheless produced a normal embryo. Hertwig ('93) has described similar cases. Fig. 5 represents an intermediate type. All gradations to perfectly equal division may be found. Something like 30 per cent of all eggs show marked deviation from equality. On the other hand, cases in which the second plane divided the egg unequally are very rare, one of which is shown in fig. 8.



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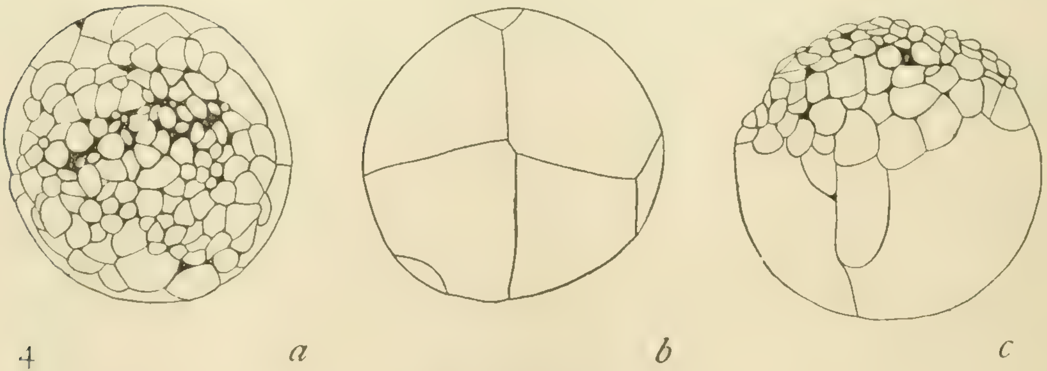
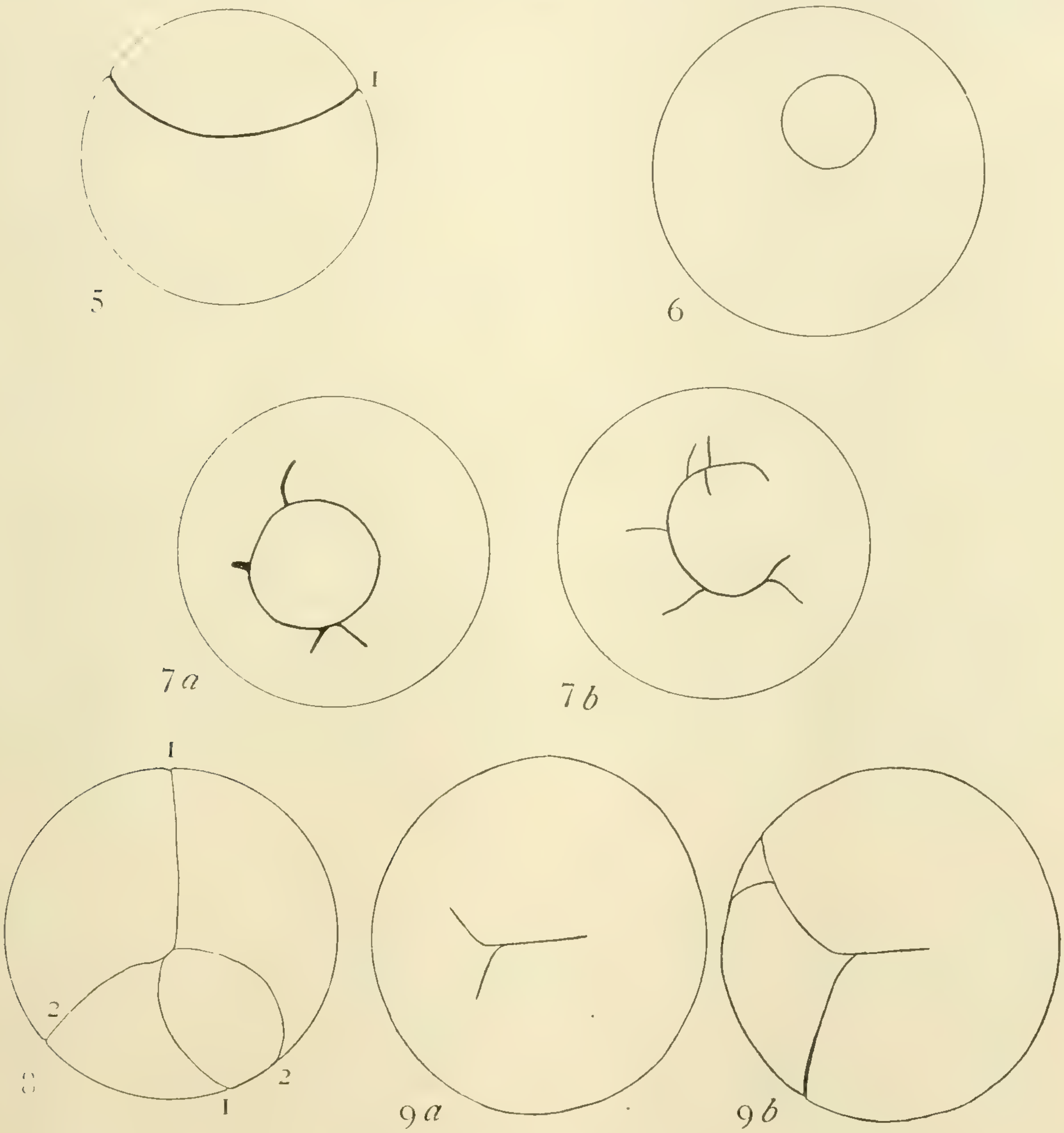


4

Figs. 3 and 4

Konopacka ('08), in centrifuged eggs, observed that the nucleus in eggs showing unequal first cleavages was displaced laterally. Doubtless this has happened in these eggs. In the egg shown in fig. 7, the formation of the second furrow was seen. Fig. 7*a* represents the beginning while fig. 7*b* shows later changes. The following day the cleavage was indistinguishable from ordinary eggs. A normal embryo finally resulted. Another type called 'Barock furchung' by Hertwig, previously noted by Born ('86), is shown in fig. 9, *b* representing a later stage of the same egg, shown at *a*. Although this egg died after developing two days, others have given normal embryos. A large number of cases of this type were seen in a bunch of eggs placed in a very small bottle before cleavage and carried about in my pocket for half



Figs. 5–9

a day. Hertwig thinks that this type results from polyspermy. It is quite possible that this lot of eggs were made polyspermic by the unfavorable conditions to which they were subjected.

Later cleavages show many irregularities, which I may not take time to describe since they differ only in minor details from other amphibians, especially those described by Rauber ('83 and '86) and Jordan and Eycleshymer ('94).

Some observations on the direction and rate of progress of the third set of cleavage furrows. The third set of cleavage furrows, preceded by a deep cavity appearing at the crossing of the first and second furrows, always start from a preëxisting furrow as is true also for all other furrows excepting of course the first. They do not first appear on the intermediate points of the blastomeres.

In general, when a furrow of the third set is horizontal, the rate of progress for the first fifth of the total distance to be travelled is slower than for the second fifth. It then tends to remain constant or to decrease for the next fifth and so on until the furrow is completed, although there are exceptions to this rule.

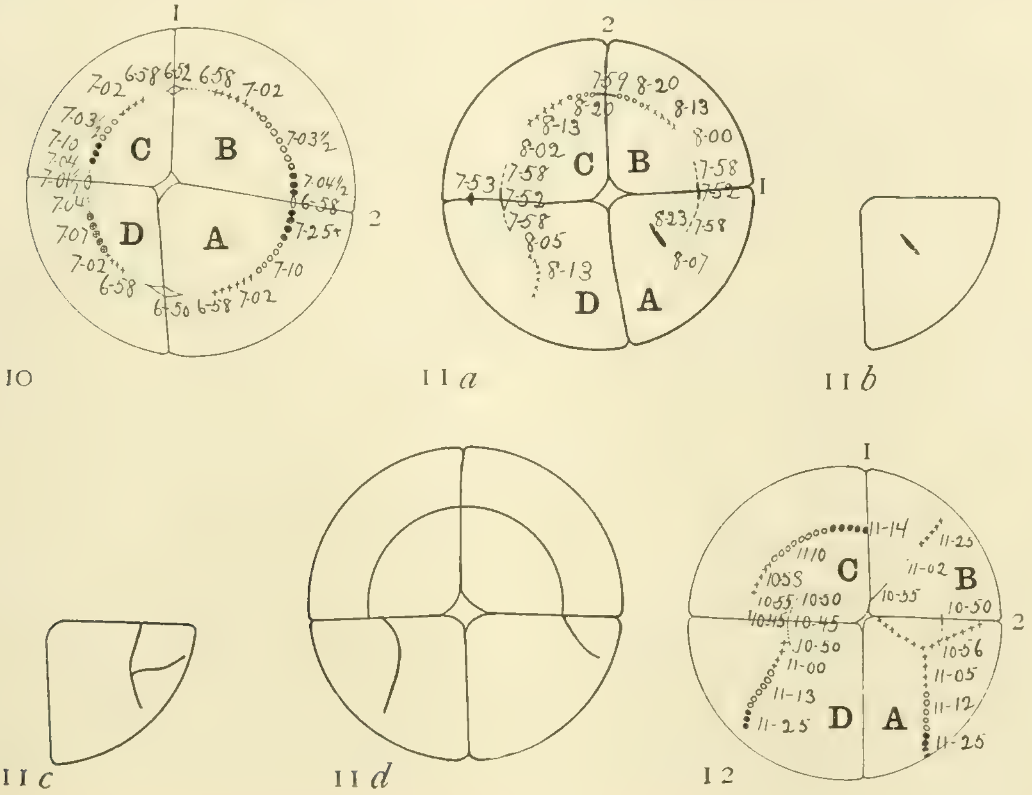
The various furrows may progress either in a clockwise or in a counterclockwise direction. Sometimes they start from both sides of a quadrant and meet at its middle. They may change from a horizontal direction to a diagonal or vertical one, or sometimes disappear, perhaps to reappear later.

DETAILS

With these general statements in mind we may take up detailed observations on several eggs. In the accompanying figures, each quadrant is designated by a letter. The cleavage furrows are distinguished by the letters of the adjoining quadrants, prefixed usually by the numeral 1 or 2, referring respectively to the first or second cleavage plane. Continuous lines indicate the first appearance of a furrow, dotted lines its next recorded extension, (in fig. 11 dashes were used instead); crosses were used for the next, circles for the next and disks for the last.

Egg no. 28, fig. 10. In quadrant *A*, the third cleavage began at 6:50 as a depression on 1 *AD* which extended into both quadrants. Eight minutes later the furrow had extended 18°, roughly speaking, into each quadrant, At this time, a minute depression appeared on the vertical 2 *AB*. Four minutes later the first furrow had extended another 18°.

Half a minute earlier, a depression had appeared on the vertical *CD* which in two and one-half minutes had extended some distance each way. At 7:07 the furrow in *D* had been completed by the union of the furrows from 1 *AD* to 2 *CD*. Returning to quadrant *A*, we find that the furrow in this blastomere had been moving more slowly. At 7:10, it reached only four-fifths of the way towards 2 *AB*. Its further progress was slow, the furrow being still incomplete at 7:25. On the vertical 1 *BC*, a depression appeared at 6:52 that is, two minutes after that on 1 *AD*. This furrow extended into both adjoining quadrants at nearly equal rates, if expressed in degrees, but actually more rapidly in *B* since the arc is larger than in *C*. After 7:02 progress of the furrow was very rapid in *B*, extending at least 25° in the next minute-and-a-half. In another minute, that is, at 7:04½ it completed itself at the depression on *AB* which had remained inactive. Meanwhile, in *C* a depression had appeared at 7:01½ on 2 *CD* as above described. Two-and-a-half minutes later it extended over about 9°. On the other side of the quadrant the furrow was progressing steadily. It was completed near 2 *CD* at 7:10 by joining the furrow already present there.



10 11 *a* 11 *b*

11 *c* 11 *d* 12

Figs. 10–12

Egg 31 *A*, fig. 11. In *C*, a furrow started on 1 *CD* and progressed entirely clockwise to 2 *CB*. In *B* a furrow started on 1 *AB* and progressed in the opposite direction to *CB*, where previously a depression had appeared. In quadrants *A* and *D* there are curious shiftings and even disappearances of furrows. In *D* cleavage started in the usual way by the formation of a pit on *CD* which gradually deepened, one part extending into *C* as already described, and the other in *D*. At 7:53 another depression appeared on the same vertical below the first. Later it disappeared. At 8:05, the first furrow in this quadrant reached over about 45°. At 8:13, it changed its direction and became vertical at its distal end. This change of direction gradually influenced the whole furrow so that it became nearly vertical. Fig. 11 *d*. Turning now to *A*, we see another remarkable series of changes. At 7:52, a depression which appeared on 1 *AB* gave rise to a furrow extending in both directions. At 8:07, the furrow extended rather more than 45° towards 2 *AD*. At 8:23, a small vertical groove appeared as indicated in fig. 11 *a*. At 8:27, the original furrow had disappeared and only the depression remained (fig. 11 *b*). This condition continued until 8:45, when the groove disappeared and in its place appeared the furrow shown in fig. 11 *c*. Later, this changed to a furrow extending towards the lower pole (fig. 11 *d*).

Egg 47, fig. 12. In quadrant *C* at 10:45, a horizontal furrow started from 2 *CD* clockwise towards 1 *CB*, which it reached in twenty-nine minutes. At the same time, a little nearer the upper pole another furrow started from the same vertical into both *C* and *D*. It extended more rapidly than the first and in five minutes had gone about 25°. The first went only 13 or 14° in ten minutes. However, the second furrow proceeded no further in *C*, but faded out. In *D* it changed its direction so as to become oblique and at 11:25 had nearly reached the equator. In *A* at 10:50, a furrow appeared on 2 *AB* and perpendicular to it, indicating the formation of a horizontal furrow. At 10:56 there was a shifting of the blastomeres in such a way that 2 *AB* became bent at its middle, *i.e.*, of the part above the equator. This point moved some distance from its original position (cf. the furrow represented by crosses). The further progress of this furrow was vertical. In *B*, a vertical furrow was formed. Shiftings of the other cells occurred but were not recorded at this time.

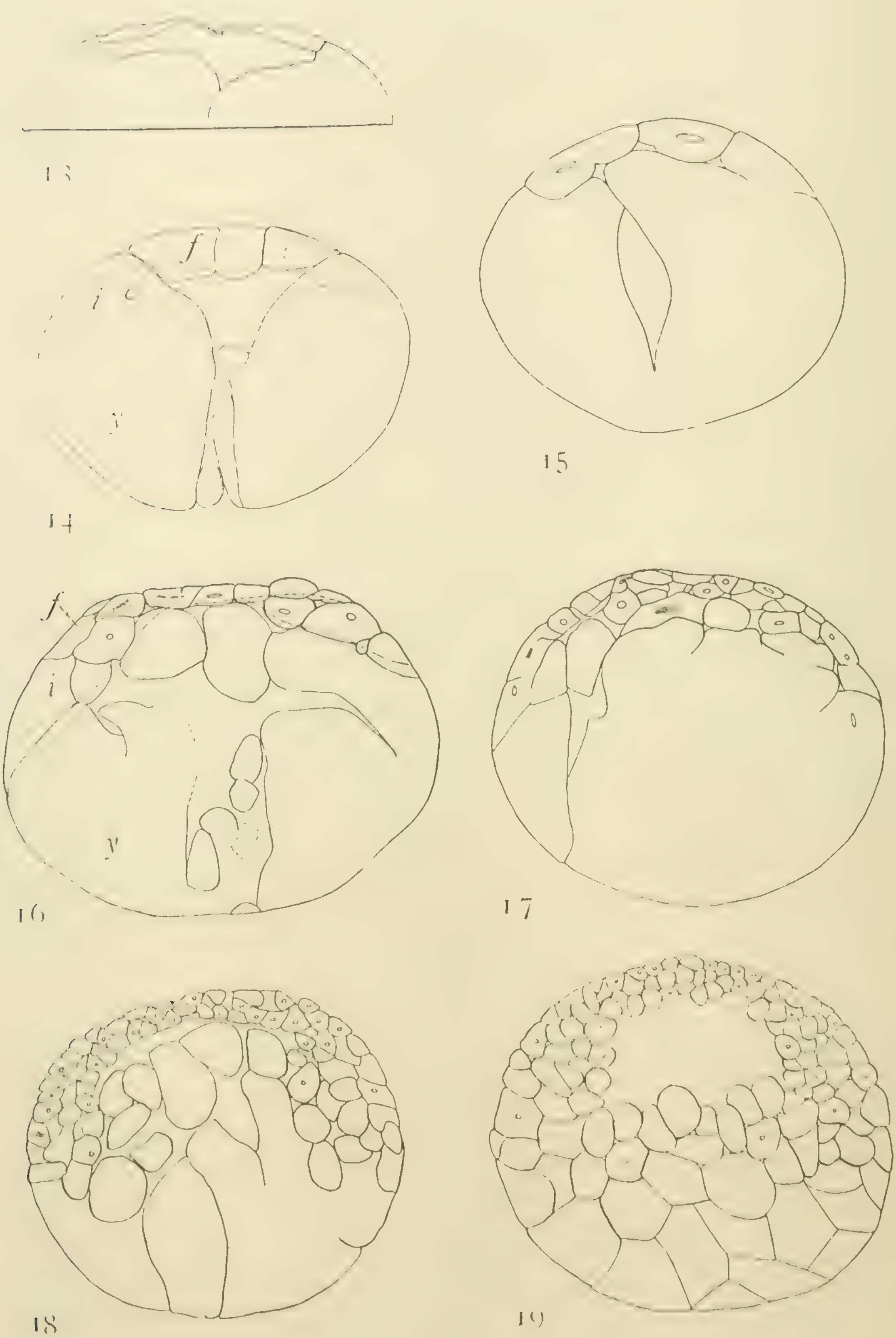
It is difficult to interpret these shiftings of furrows. It points towards a lack of complete control by the nucleus over cell division. The latter may initiate cell division but the cytoplasm would seem to control its further progress. Probably the deflections are produced in part by differences in the constitution of the yolk. If, for example, there were more yolk granules in one spot and therefore less protoplasm, the planes of cell division might more easily follow the more protoplasmic parts of the egg which lay to one side of its projected path. It is evident, however, that speculation in the subject is quite idle for the present. In some of the early cleavage stages, I have accidentally deflected the course of a furrow. If a spot of stain (described below) be placed on an egg in the path of the advancing furrow, the furrow does not always pass through the spot but may curve around it, following its edges closely just as though the stained spot were a real obstacle which it could not overcome. Such cases lend favor to the interpretation suggested above.

Internal aspect of cleavage

The differences among individual eggs make it difficult to give a satisfactory account of the process of cleavage within the egg. Variations are so numerous that I shall describe only such as seem necessary to show its usual course.

The plane of first cleavage as it cuts into the egg may separate the two halves of the egg material by a considerable space. With further progress, the space closes up by the approximation of its edges as already described. The parts of the egg already cut, that is, the deepest parts of the furrow, remain separated for a time, so that when an egg is sectioned perpendicularly to the first furrow in a vertical plane, a sort of cleavage cavity is formed. But this is only temporary. In some cases at any rate, the edges of the cells come together and *actually fuse.* A careful study of the sections of an egg similar to the one shown in plate I, fig. 2, from which it differed in showing no furrow whatever except in regions corresponding to the spoon-shaped ends of the furrow, failed to show any separation of egg material except at points where a furrow was visible externally. The lower part of the yolk mass may not be cut through by the cleavage plane for a long time. The progress of the second plane is similar to the first.

In some eggs at the eight-cell stage, those parts of the micromeres which lie at the upper pole of the egg become very thin (fig. 13), although they are thicker towards the equator. A vertical section of another egg at the eight-cell stage, cut diagonally to both of the first two planes of cleavage, is shown in fig. 14. It represents the other extreme in variation of micromere shape. In later stages, this last is the common type, only occa-



Figs. 13–19

sional triangular cells being found. At other times, the cells are conspicuously lobed as seen in the yolk cells of figs. 14 and 16. The same thing at a sixteen-cell stage is shown in figs. 20 and 21. In still another type, where no cleavage cavity at all is formed, the micromeres lie directly upon the macromeres. When present, the cleavage cavity may be large as in fig. 14, or quite small as in figs. 20 and 21, where it is represented by intercellular spaces.

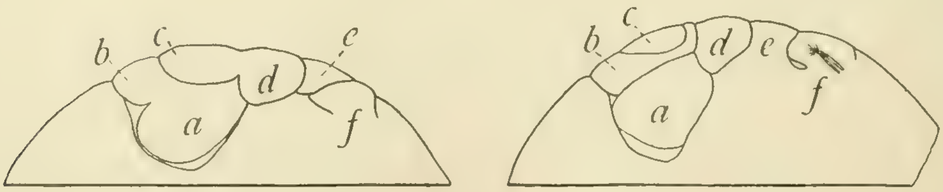
As cleavage proceeds, a condition develops similar to that figured by Grönroos ('95) for Salamandra maculosa. There is an outer layer of cells upon the upper hemisphere and lying within this, a second series concentric with the first (figs. 16–17). The cells of the outer layer are smaller, more angular and, for the most part, contain fine yolk-granules, although sometimes their inner ends contain coarse granules. The inner layer has large, rounded cells with coarse yolk granules except that occasionally finer granules occur in their outer ends (fig. 16). This condition arises in the following manner: At the twelve-cell stage there is a rather thin cap of eight cells lying on the surface of the egg (fig. 15), which has arisen by division of the four small blastomeres of the eight-celled stage. The remainder of the egg shows no sign of division except those of earlier planes. After the next few divisions, the yolk mass has usually given off one to four large rounded cells, which lie above the center of the egg. By repeated divisions of the cells already formed and the addition of cells thereto from the lateral parts of the yolk, both layers increase in size (figs. 17 and 18). There is often no sharp boundary between the two layers (fig. 17). Sometimes the lower parts of the cells belonging to the outer layer are added to the inner in the manner described by Reed ('05) for the frog. Quite often one or two cells are much larger than the rest of the inner layer. Similar cells or their descendants may often be seen until a comparatively late period (fig. 18).

During this time, the cleavage cavity has existed for the most part as intercellular spaces, although sometimes it is well developed. Then a well-defined outer layer appears (fig. 18). The next step, presumably, is that those yolk cells which do not enter into the formation of the upper layer of cells, sink down, resulting in the formation of a well-defined blastocoele (fig. 19). In some blastulae the roof consists of only two layers of cells while in others they are more numerous. The smaller eggs as a rule appear to have the thicker roof. The fluid of the blastocoele contains some substances, probably albuminous, which, on fixing, appear as a white, rather flocculent mass.

Often the cleavage planes cut only the more superficial portions of the egg at first and only later cut through the interior. This is shown in figs. 16, 17, and 18. The interior of the egg, lying below the level of the equator, often is the last to be divided into cells.

Connected blastomeres

It is not certain whether the connected blastomeres here described, result from a non-development of the cell wall or from a fusion of cells. Fig. 20 shows a vertical section of an egg which externally appeared to be composed of sixteen cells. Fig. 21 shows the fifth section beyond. Corresponding parts of the egg have been given the same letter. In fig. 20, *a* and *b* appear as part of one cell, as do *c* and *d*, while *f* is connected to the general yolk mass; *e* is a separate cell. In fig. 21, *a*, *b*, *c*, and *d*, are all separate cells, while *e* now joins the yolk mass. Study of intervening sections show that fig. 20 represents a true condition. Careful study by means of camera drawings of all the sections of two eggs showed that while twelve (possibly thirteen) nuclei were present, only two completely separated parts existed. The area between two cells lacking a cell-wall was often small, but was definitely present. In other eggs, however, only slight evidences of connected blastomeres were present.



20 21

Figs. 20 and 21

The objection may be raised that the preserved material does not represent actual conditions in the living egg, but there are several arguments against such a point of view. In the first place, it is well known that it is very difficult to separate amphibian blastomeres. They tend to disintegrate after separation, which indicates some sort of continuity. In the second place, material fixed in some of the picric mixtures, which have a tendency to *separate* the blastomeres, show the same phenomena. The cell-walls when present are perfectly distinct except in a zone of transition leading to the region where the egg material is still unseparated. Third, there is no evidence that the cell-wall had previously existed and been torn from its place in sectioning. The contents of one cell simply pass without any break or interruption of any sort into the next.

Changes in the size of eggs

Often when a group of eggs is examined as they lie upon the stone to which they are attached, it is noticed at once that some of the eggs are considerably larger than others. Close examination shows that the largest eggs have a translucent upper hemisphere, indicating an advanced stage of development. Besides this, eggs in the same cleavage stages often differ much in size. Observations made on a very few eggs indicate, first, that the relative size differences are maintained throughout the early course of development, and, second, that after the first day, there is a gradual increase in the size of the eggs until after the close of gastrulation, when a slight decrease occurs. An extended series of observations was not made, the object of this series being to determine somewhat more accurately than by mere inspection, the time and amounts of increase in size of the eggs.

Four eggs were taken, two 'large,' and two 'small.' Careful camera drawings of the outlines of the eggs were made at intervals, usually about a day apart. Since the outlines as drawn by the camera are oval, the measurements in the following table were made on the shorter axis. Variations in focusing have made slight differences in the records. The units in table 1 are arbitrary. They nearly equal $\frac{1}{8}$ mm.

TABLE 1

Showing changes in size of eggs

DATE	DIAMETER OF SHORT AXIS OF SKETCH				REMARKS
	Large		Small		
May 11 3 P.M....	20	19½	17½	17	Before eggs had divided
May 12 5:15 P.M..	20	19½	17	16½	
May 13 8:30 A.M..	21	21	18½	18	
May 13 7 P.M.....	21−	21−	18	18	
May 14 8 A.M.....	23	22	19	19	
May 15 9 A.M	23	22½	20½	20	Three eggs gastrulating
May 16 8 A.M.....	23	22	18	17	One died. Room temp. 25° C.
May 17 2 P.M.....	25 (22)*	22½(20)*	18	18	Neural folds. Temp. 25° C.

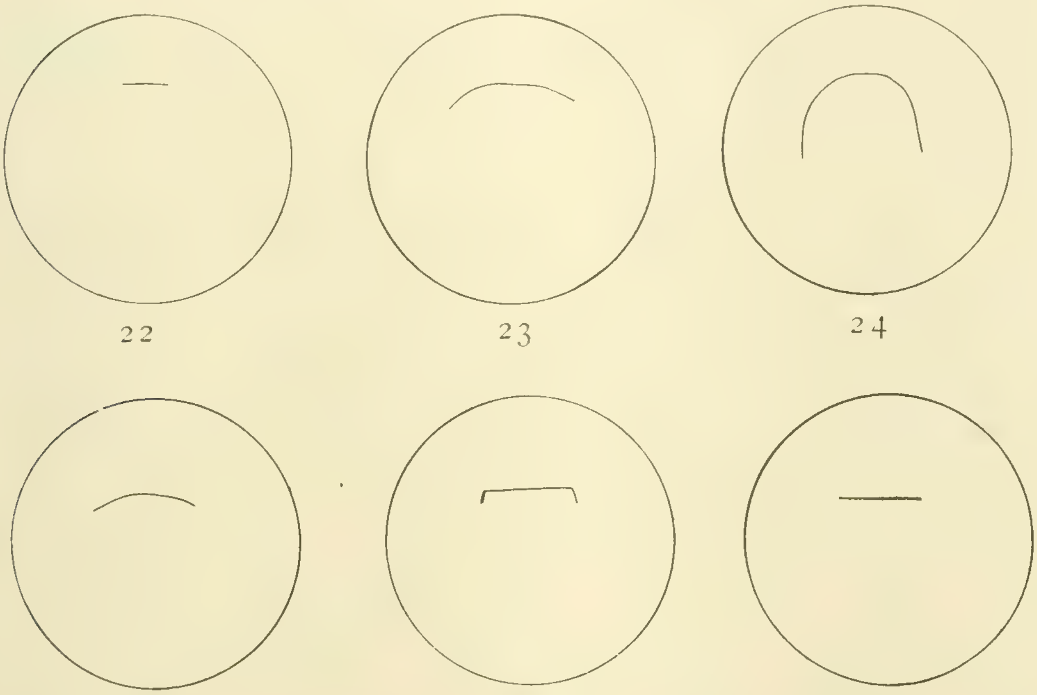
* Diameter of an egg from another set of 'large' eggs, which, being in a larger dish were not affected by the high temperature prevailing.

These measurements show, in addition to the results already mentioned, that the eggs are largest during gastrulation. The fluid filling the enlarged internal cavities of the egg appears to be derived from the perivitelline space which does not share in the increase in size, for the surface of the egg at this stage is separated by a smaller distance from its membranes than was the case earlier.

Changes in the shape of the blastopore and its fate

The first appearance of the blastopore is a very shallow, narrow cleft appearing among the yolk cells some 30–45° below the equator (fig. 22). It elongates rapidly and becomes crescentic (fig. 23). The concavity of the crescent is directed towards the lower pole. The dorsal lip appears to be nearly stationary. The elongation of the blastopore continues until it corresponds in extreme cases to a little more than half a circumference (fig. 24) though often it does not attain so great an extension. The blastopore has not moved much, if any, from the point where it originated towards the lower pole, differing in this respect from the blastopore of the frog.

During this period, the cells at both sides of the blastopore have been invaginating, until, dorsal to the lip, they are quite small, but ventrally are still large. Although the movement of cells into the blastopore continues ventrally it appears to cease above the blastopore, which gradually shortens until it again becomes a small crescent, (fig. 25), very similar to the crescent of earlier stages. The difference in size of the cells bordering the blastopore is the only feature by which this stage can be distinguished from the similar earlier stage. Very soon the neural groove appears which at once serves to orient the egg. It will be noted that no ventral lip was formed.



Figs. 22-27[5]

A slight rotation of the egg about a transverse horizontal axis occurs at this time, bringing the blastopore up so that it lies just beneath the equator.

The blastopore now loses its crescentic shape by straightening out into a horizontal groove except at its ends, which remain bent downwards almost at right angles with the rest of the blastopore (fig. 26). The ends soon shrink away, leaving the blastopore as a straight horizontal groove at the posterior end of the neural plate (fig. 27). As the neural folds begin to develop, the blastopore continues to shorten and at the same time again becomes crescentic, but this time, its concavity is directed dorsally, exactly opposite to its previous direction, plate 1, figs. 8 and 10. The blastopore continues to shorten, sometimes becoming a short thick, horizontal opening, or a short thick vertical slit (plate 1, fig. 12), or even other shapes. At last however, it becomes a small rounded opening at the ends of the neural folds, where it remains as the definitive anus.

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[5] Figs. 22 to 27 are diagrammatic.

The numerous investigators into the fate of the amphibian blastopore have failed to agree, but it appears that in most Anura, the anterior part of the blastopore, without closing becomes the neurenteric canal, while the rest of the blastopore closes up. The anus arises as a new invagination through the posterior part of the fused space. In some Urodeles, *e. g.*, Amblystoma, the median parts of the slit-like blastopore fuse leaving an anterior opening, the neuropore, and a posterior, the anus. In others, *e.g.*, Sperlepes, in which there appears to be no neurenteric canal, the blastopore becomes the anus.

Formation and closure of the neural folds

About the time the blastopore becomes a straight line, the neural groove appears. At first it is a wide, short and shallow depression, just posterior to the present upper pole of the egg. It is, then, near the original primary equator of the egg. It gradually deepens and then lengthens, until it forms a furrow, sometimes deep, sometimes shallow, extending over about 90°. Its posterior end is usually near the equator. Sometimes it is continued by a dark stripe to the blastopore.

The first appearance of the neural folds as two ridges, one on each side near the equator, is shown in plate 1, fig. 7. Their outer edges coincide with the boundaries of the neural plate which can be distinguished as an elongated, slightly irregular ovoid. The neural groove in this particular egg is narrow and extends from a point near the anterior end of the neural plate back towards the blastopore, plate 1, figs. 7 and 8. At its point of origin, it is slightly deeper than in the remaining parts.

Plate 1, figs. 9 and 10. The neural region is slightly elevated anteriorly and is distinctly differentiated throughout its entire extent. At the anterior border, the neural folds are not yet raised above the general surface of the egg, although they can be made out without much difficulty. In the lateral regions, they begin to ascend gradually as two distinct ridges, now considerably broader than before, reaching their highest point anterior to the middle of the neural area. Posterior to this point, they gradually descend until only faintly discernible. In this particular egg, they appear to surround the blastopore. In other eggs, this fact is not evident. The usual further development of the folds is well shown by figs. 11–14, plate 1, without comment. Sometimes the folds remain parallel while closing.

When the neural folds are nearly closed (fig. 13, plate 1), a slow rotation of the embryo about its horizontal, longitudinal axis begins, so that in the course of a few hours the embryo lies on its side and often on its back. Later it rotates constantly about a vertical or oblique axis, sometimes slowly, sometimes rapidly. It may rotate head or tail foremost. This rotation is presumably due to cilia, although I have never seen them. Such rotation of amphibian embryos has been noted by Clark ('80), Eycleshymer ('95), Assheton ('96) and Wilson ('97).

From the time the neural folds become well-defined throughout their entire extent, the embryo rapidly elongates, so that when the folds have closed, it extends over 315°, being curled ventrally about the yolk cells. The head and tail are thus brought close together. While the question of elongation has not been carefully studied, there is some evidence to show that the greater part of this elongation takes place in the posterior part of the animal. In several cases among the stained eggs to be described later, marks made in the posterior regions have lengthened. In the anterior regions, on the contrary, such elongation has not been noted.

These marked eggs show also that the neural folds close by an actual transference of the neural plate material and not by a wave-like elevation and movement of cells. It will be recalled that the first appearance of the neural folds is near the equator. A stained spot in this region, *i.e.*, adjoining the epidermal ectoderm, moves bodily to the dorsal mid-line. This is confirmed by sections, for the material of the neural plate ectoderm is composed of fine yolk granules, while the cells of the epidermal ectoderm are filled with much larger granules. When the neural folds have closed, the cells containing the large granules from either side have met in the dorsal mid-line. They now cover the entire outer surface of the animal, except, perhaps, a small area immediately in front of the head. Nearly all the material of the neural plate, then, goes to form the nervous system of the adult.

Discussion of the neural groove

The shallow median groove described in previous sections constitutes the so-called neural, median or dorsal groove. Some authors apply the term neural or medullary groove to the entire space separating the neural folds. Cross-sections of the neural groove in early stages, show that in this region the ectoderm is much thinner than at either side and is composed of a single layer of cells which may be described as rounded, cubical. It lies directly over the notochord which here forms the dorsal roof of the archenteron. *The true significance of this groove appears to be entirely unknown.* Hertwig ('92), Van Bambeke ('93), Braus ('94, '02), Rothig ('01) consider it as the seam along which the blastopore lips fused during the concrescence of the embryo. Brauer ('97) points out that in Hypogeophis, in which there is very little movement of the blastopore ventrally and in which the embryo is well developed long before the blastopore closes, it cannot represent such a seam. Schultze ('83, '88), Johnson ('84), Morgan ('89), Erlanger ('90), have considered it, either in whole or in part, as the primitive streak. Goette ('75) believes the neural groove is due to the slower development of the notochord, which fails to support the middle of the neural plate, thus permitting this part to sag. Schwink ('84), Erlanger ('91), Robinson and Assheton ('91), Jordan ('93), Eycleshymer ('95) note the presence of the groove, but do not attempt to assign it any significance. They consider that the very short line of real fusion of the blastopore lips, which takes place after the blastopore has become a vertical slit, is the primitive streak, since here all three primary germ layers are fused. Altogether there is much confusion on the subject, but I believe we may safely dismiss the view of the first two groups of authors as untenable; that of the first because of the lack of proof of concrescence and that of the second because there is no fusion of the three germ layers along the median groove, which fusion is now the generally accepted criterion of the presence of a primitive streak.

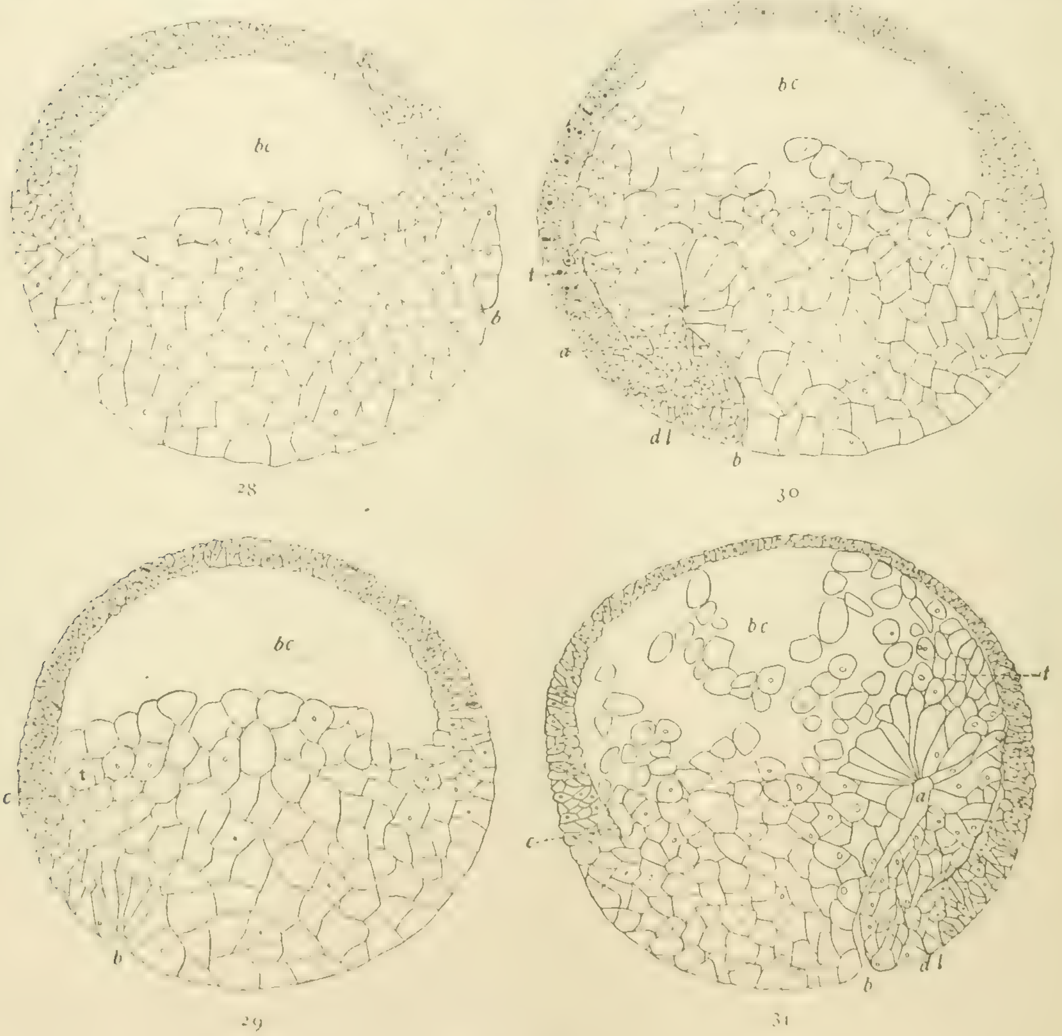
Gastrulation

Just before the egg is ready to gastrulate, a vertical section (cf. fig. 28, which represents a slightly later stage) shows a well-developed blastocoele, *bc*, whose nearly level floor passes without break into the side walls. Its roof at the upper pole contains one or two layers of cells with small granules. It thickens downwards to three or four layers of large cells whose granules are of intermediate size. The yolk cells are polygonal, except where rounded next the blastocoele.

A vertical section of an early gastrula is shown at fig. 28; the corresponding external view in fig. 22. The blastopore, *b*, a narrow groove (cleft or split) lies about 30° below the equator among the yolk cells, which are triangular at the bottom of the groove. They appear to be pulling away from the surface as has been noted in other Amphibians.

A little later (fig. 29, cf. fig. 23), there are numerous triangular cells about the blastopore whose long axes are all directed dorso-ventrally. In the living egg they are flask- or bottle-shaped with very long necks. The floor cells of the blastocoele are now separating from one another, while the floor itself is arched. Over the blastopore, it is raised into a tongue, *t*, leaving a cleft, *c*, between it and the outer cells as noted by H. V. Wilson and others.

Fig. 30. The groove has deepened into a slit-like archenteron, *a*, around whose inner ends are the elongated triangular cells. The tongue, *t*, is larger, while the cells of the dorsal lip, *d. l.*, are smaller and more numerous. The blastopore has lowered to an amount equal to the depth of the archenteron. As I shall show by means of artificial stained spots on the egg, the small cells now lying at the edge of the blastopore are derived by division from cells which lay at the equator at an earlier stage. The roof[6] of the blastocoele is thinner, especially above the blastopore. Numerous mitotic figures are present just above the blastopore. The floor-cells of the blastocoele are migrating singly or in groups towards the roof against which they place themselves. This



28 30 29 31

Figs. 28–31

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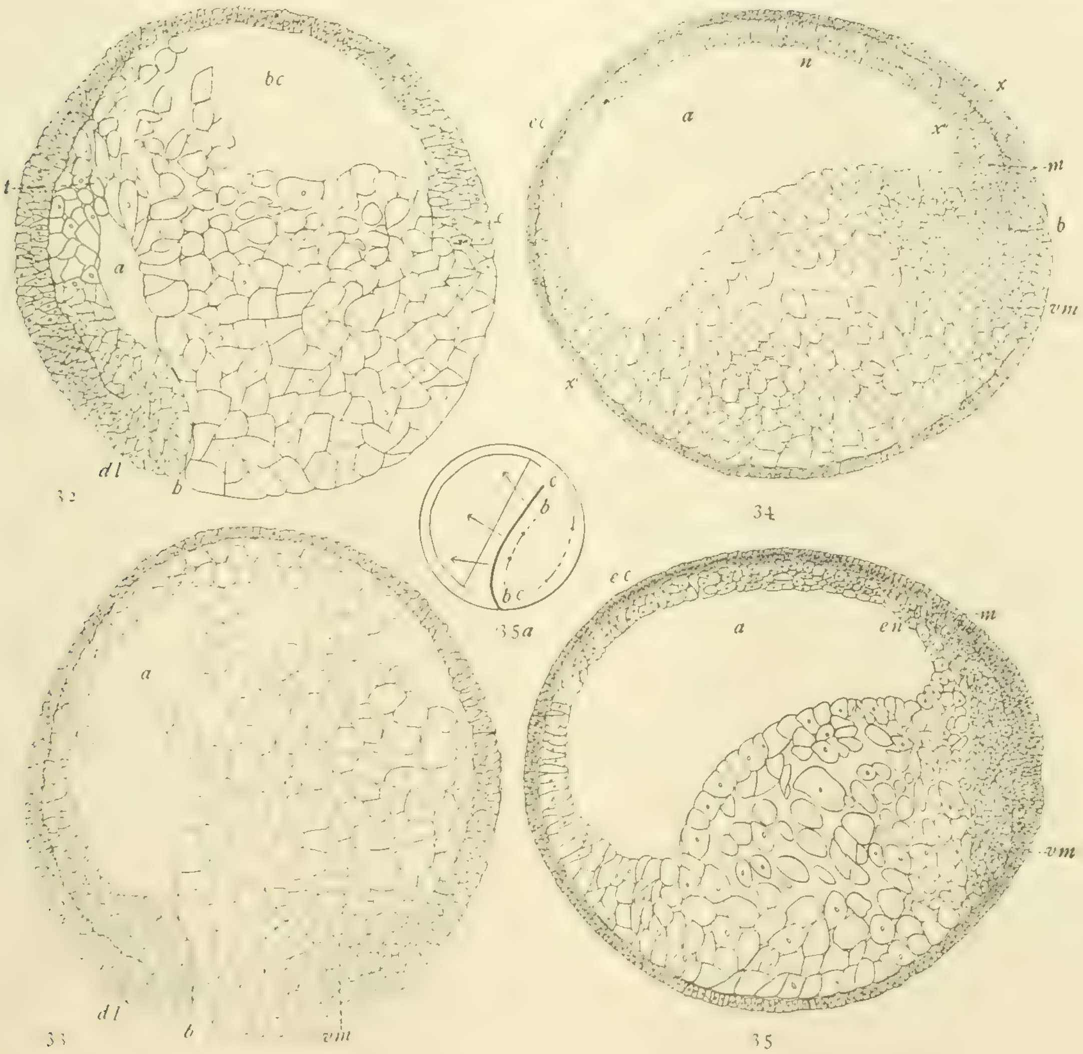
[6] The roof of the egg shown in fig. 30 is unusually thick.

migration of yolk-cells, disregarded in recent years, appears, according to Moquin-Tandon ('76), to have been first noticed by Stricker in a paper I have not seen. In fig. 31, the blastocoele is filled with migrating cells, which cause the surface of the living egg to appear mottled. The archenteron has become enlarged but there has been little or no more invagination at the dorsal lip. Opposite it, a short cleft, *c*, has appeared while the exposed surface of the yolk has decreased. Fig. 32 is drawn from an egg of the same age as fig. 31. It is more developed in some parts although less in others.



Figs. 32–35

Gastrulation, or better, invagination is nearly completed in the egg shown in fig. 33. The ectoderm, reduced to a single layer of cells above, is still several layers thick at the blastopore. The anterior part of the archenteric roof is formed of large yolk cells, which become smaller posteriorly, although their granules remain large. Close to the lip, granules of intermediate size are found. Not far below the blastopore, the ventral mesoderm, *vm*, fig. 33, can be seen among the yolk cells which are now almost entirely invaginated.

Finally, just before the embryo appears, the formation of the archenteron is complete (fig. 34, cf. fig. 26). The ectoderm cells are smaller and arranged in a single layer except around the blastopore. The triangular mass of ventral mesoderm, *vm*, lies just below the blastopore. The columnar yolk cells in the posterior part of the roof of the archenteron, are clearly notochordal. Anteriorly, they have not yet differentiated. The cells marked *m* in the dorsal wall near the blastopore are probably mesoderm.

Fig. 35 is a lateral section of the same egg, passing through the end of the transverse blastopore. The posterior part of the roof of the archenteron has differentiated into the endoderm, *en* and mesoderm, *m*, the last fusing at the blastopore with the ectoderm. Near the middle of the roof, mesoderm and endoderm join the still incompletely differentiated cells of the anterior half.

The yolk granules of the dorsal ectoderm cells (fig. 34), lying between x and x', are of the smallest size. Between x' and b, as well as in the dorsal lip between x and x'' are the intermediate granules, while the large ones are found in the rest of the egg.

Mechanics of gastrulation. I shall not attempt to go into this question extensively, but will merely state the conclusions to which my observations and experiments lead me. Since the yolk-cells can be seen actively migrating at certain stages and since it can be shown that the cells of the equatorial band divide and migrate, I conclude that the yolk cells and the cells of the Randzone are in a condition of active movement. Neither kind moves the other passively, but each is actively moving in coördination with the other to its proper place. I cannot accept the view that gastrulation is due primarily to increased cell-division in certain places, or to increased growth of local areas of cells which cause other areas to invaginate. The cause of gastrulation is an inherent property of the cells of the egg, especially the yolk-cells, just as much as it is of ectoderm cells to form the neural structures. It explains nothing to make this statement, but it compels us to look further, if we wish to understand the mechanics of gastrulation.

Thinning of blastopore roof. The roof of the blastopore constantly becomes thinner from late cleavage until the end of gastrulation. The increase in size of the egg alone during this time will not accommodate the increased surface provided by the thinning ectoderm. Stains made on the upper surface of the egg in its central half show a constant tendency to increase in area by spreading centrifugally, due, probably, to a re-arrangement of its cells, since stained spots near the equator become elongated into definite bands, indicating a division of cells followed by migration of part of the daughter cells.[7] When the egg is largest, the upper hemisphere, now translucent, slightly overhangs the lower, that is, its radius is slightly larger. The necessary surface for this increase is, I believe, furnished by the thinning central half of the upper hemisphere which forms approximately one-fourth the entire surface of the egg. Occasionally, an egg fails to gastrulate, but becomes very large. Such eggs have unusually thin roofs.

The thinning of the outer half of the upper hemisphere provides the necessary ectoderm for the ventral hemisphere. As the central half of the upper hemisphere, containing the small granules which are to form the embryo, remains within the upper hemisphere, the embryonic material (in narrower sense) is not carried below the equator, as in the frog. Consequently, the embryo differentiates largely in the upper hemisphere.

Very often the middle half of the upper hemisphere is translucent during some stages of gastrulation. In some cases a shadowy area can be seen projecting upwards above the blastopore into the translucent area as described by Ikeda ('02) for Rhacophorus. This shadowy area has been noted to increase. Its shape is exactly what one would expect if it were the tongue of cells about the archenteron, shown in figs. 30–33. But unfortunately for this expectation, in other cases this shadowy area may lie on the side opposite to the blastopore or even half way between these points. Sometimes, small shadowy spots appear scattered over the translucent area. These I have already interpreted as migrating cells. The appearance of a shadowy area opposite the blastopore may be explained perhaps by reference to fig. 33. The yolk cells at the anterior end of the archenteron, when looked down upon in the living egg, would appear opaque compared to the rest of the dorsal side, which, although increased by the addition of the cells of the roof of the archenteron, is actually very thin. These shadowy areas are then undoubtedly connected with the process of gastrulation, but their exact relation to the various phases of gastrulation needs further investigation.

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[7] For method, etc., see page 209.

The origin of the roof of the archenteron. I wish now to discuss the sources from which the cells of the roof of the archenteron are derived. From the history of the yolk cells during gastrulation, as well as from a study of the yolk granules given on page 178 it is evident that the greater part of the roof of the archenteron is formed from the yolk cells. Even the posterior part of the roof is derived from yolk cells, if by such we mean the lower four cells at the eight-cell stage. If they were from animal cells they should have small granules. Moreover, the upper four cells of the eight-cell stage lie above the region of cell migration (see page 224 et seq.). I have endeavored by means of stains as described below, to determine the amount of invagination of the cells which lie above the blastopore when first formed, but thus far I have been unable to do so satisfactorily. However, by breaking open a marked egg preserved in the chromic-acetic acid mixture, I find that the stain when applied to the dorsal lip appears to be invaginated to the amount already indicated by the yolk granules (fig. 34, x'').

The cells dorsal to the blastopore lip when first formed (figs. 28, 29) come to form part of the archenteric roof. Do the cells ventral to the lip also come to lie in the roof of the archenteron, or do they form some other part of the archenteron, perhaps the anterior part of the floor?

In the first case, we may suppose that they pull up and roll around the end of the archenteron (figs. 30–32) and so come to lie in its roof; or, in the second case, we may suppose that they do not roll around the end, but maintain their position in the floor of the archenteron and are carried forward by the extension of the archenteron to their final position at its anterior end, that is, from *b* to *b′*, fig. 35*a*. The roof of the archenteron may be supposed to be formed by a process of splitting and rearrangement of the floor cells of the blastocoele, part of which move in the direction of the large arrows in fig. 35*a*. Others, especially those near *b′* and *c*, may move in the direction *c* to *c′* and come to lie next the ventral ectoderm. Some of the cells beneath the archenteric split may even come to form part of the anterior roof of the archenteron. Those yolk cells forming the outer layer of the ventral hemisphere of the blastula (fig. 35*a*, *c*-*c′*), then, would come to form much of the floor of the archenteron, since definite proof is given below that these cells actively invaginate. If we could superimpose the roof of the completed archenteron as shown in a vertical section, onto a similar section of the blastula, it would appear much as in fig. 35*a*. It is obvious that if the cells at *b* move to *b′*, the cells between *c* and *b* would form much of the floor. If, however, the first case suggested is true, then the roof of the archenteron has a mixed origin, part of its cells being those which earlier formed the floor of the blastula and part external yolk cells. Its floor, on the other hand, is formed in a different way from that suggested for the second case, its posterior part being formed from external cells near *c*, and the remainder being derived by a process of splitting from the yolk cells of the interior. At present, conclusive evidence, bearing on these suggestions, is wanting.

The mode of origin of the archenteric roof has been a source of much discussion, due partly to actual differences among the Amphibia, and partly to differences of opinion as to the value of the evidence opposed. Thus, some believe that the presence of pigment is proper evidence, while others deny this.

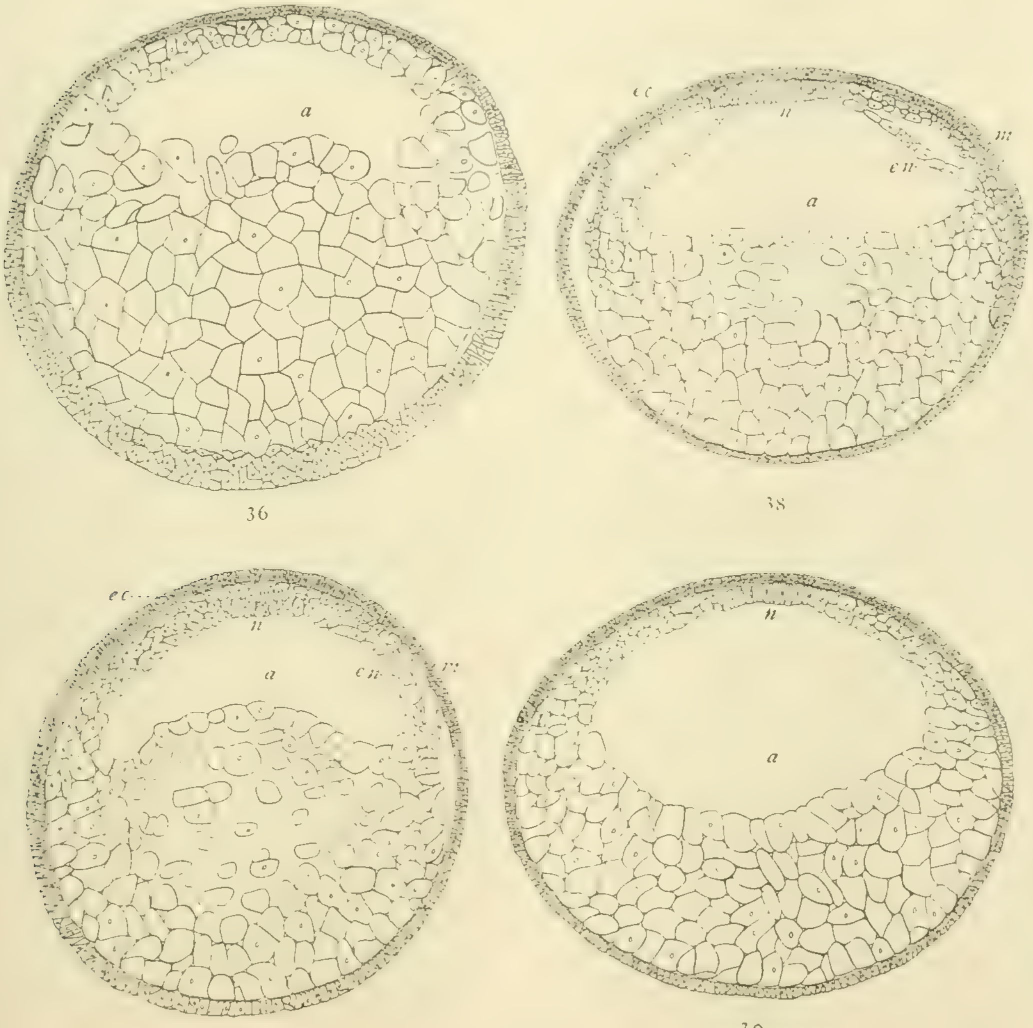
For the most part, it is generally conceded that some part of the archenteric roof is formed by an invagination of those cells which lie above the dorsal lip at its first appearance. Whether they be called ectoderm, animal cells or what not, is of no importance for the present, though most authors would doubtless call them ectoderm. As to the source of the rest of the archenteric roof, there is relatively little agreement, as shown by the papers of Stricker '62, Goette '75, Moquin-Tandon '76, Hertwig '83 and '03, Schultze '88, Schwink '89, Houssay '90, Robinson and Assheton '91, Jordan '93, Lwoff '94, Morgan and Tsuda '94, Eycleshymer '95, Samassa '95, Brauer '97, Grönroos '97, Wilson '00, Adler '01, King '01, Ikeda '02, Morgan '02, Brachet '03 and Hilton '09.

Mesoderm

The mode of origin of the gastral mesoderm in Spelerpes is perfectly clear, although it apparently appears in some eggs at an earlier stage of development than in others. The youngest embryo I have, in which there is any trace of mesoderm is one in which the archenteric invagination has reached the stage shown in fig. 33. A cross-section of such an egg through the blastopore region shows that the peristomal mesoderm, composed of small polygonal cells, forms the roof, sides and floor of the archenteron. Anteriorly, the archenteron roof consists of several layers of small cells, which extend slightly below the level of the floor of the archenteron. This extension of small cells I take to be precociously differentiated mesoderm. However, there is only one egg in which I have seen this phenomenon so clearly at this period.

Cross sections of slightly older stages usually fail to show a similar extension of small cells, although the cells of this region may not be quite as large as the neighboring yolk cells, which are usually separated from one another (fig. 36). The separation of cells is merely an expression of their migration. At this time, the cells in the dorsal region have again arranged themselves into a definite layer of irregular yolk cells, but at the sides, where invagination started later, this re-arrangement is not yet complete.

The section just mentioned is the 61st from the blastopore end of the series, in a total of 106 sections. It is not quite vertical, the ventral part lying posterior to a vertical plane, the dorsal part, anterior. The figure represents the condition of the entire roof of the archenteron except in the immediate region of the blastopore, where the archenteron is bounded dorsally and laterally by rather small cells, as might be expected from an examination of fig. 33.



36 38 37 39

Figs. 36–39

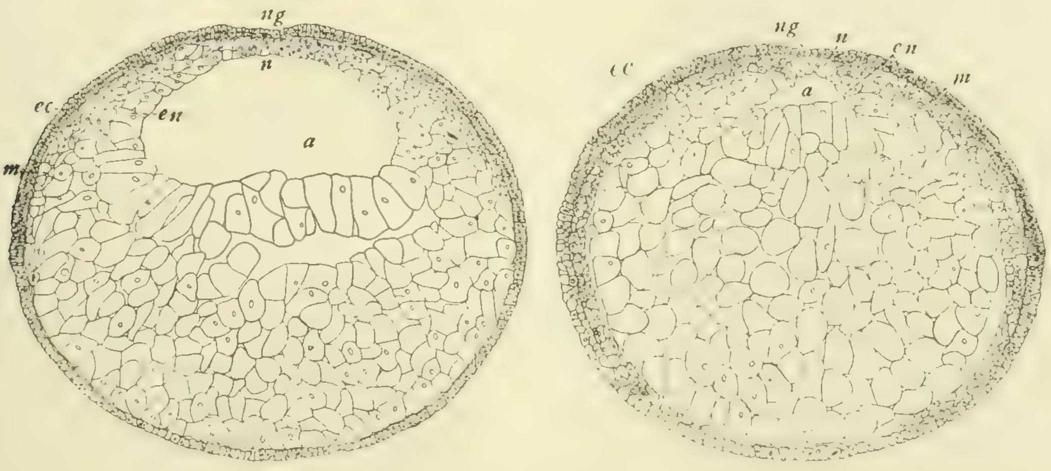
Not until the archenteric pouch has reached its greatest extension and the cleavage cavity become practically obliterated can we definitely separate the yolk cells of the lateral roof of the archenteron into mesoderm and endoderm. The former is a layer of small cells (fig. 37), usually two deep, but not yet forming well-defined layers. It extends from near the mid-dorsal region to near the level of the floor of the archenteron, *a*. The endoderm cells, *en*, are larger and usually arranged in a single layer. In the mid-dorsal region, both endoderm and mesoderm graduate insensibly into a mass of indifferent cells, arranged in three or four layers and about the size of the mesoderm cells. Its middle part is the forerunner of the notochord, *n*, while the cells at the side may become either mesoderm, endoderm, or chorda. This arrangement exists throughout the posterior half of the embryo. Anteriorly, the cells are still undifferentiated.

The section figured is the 36th from the blastopore in the series of 106. The mesoderm can be made out up to the 60th section. On the left side of the drawing, the mesoderm is not so distinctly differentiated. In the actual sections the cell walls of the mesoderm cells are as a rule better developed and hence more sharply stained than those of the endoderm. This distinction is naturally lost in the drawings.

The differentiation of the yolk cells of the archenteron into mesoderm and endoderm, which, it must be admitted, is a little vague in fig. 37, is fully verified in the next, fig. 38, which represents the 30th section from the posterior end of the egg. The mesoderm can be followed to the 60th section. Its cells, *m*, are rounded, much smaller than the endoderm cells and usually form a double row. The latter's cells, *en*, are somewhat elongated and attached end to end. As shown in the figure, this layer as a whole is easily pulled away from the mesoderm in sectioning. This frequently happens and always in the same manner, indicating differences in cohesion of the various layers. The cells of the mid-dorsal region, *n*, have become columnar and clearly form the anlage of the notochord. At either side of the notochord the cells are irregular and all three kinds, viz.: notochord, mesoderm and endoderm are fused together. At the sides below the level of the floor of the archenteron, the mesoderm cells can be followed for a short distance. On the right side in one place, they are not much different from the neighboring yolk cells from which they are probably differentiating. There is a large archenteron, *a*, which is even larger in the anterior regions (cf. fig. 39, which, while not drawn from the same egg, is from an egg at the same point of development. This section is the 63rd in 106).

The notochord cells, *n*, are distinctly differentiated, but in the lateral roof, the differentiation into mesoderm and endoderm is scarcely discernible in sections and does not show in the figures.

Fig. 40, the 56th section in 105, shows an advance in the differentiation of germ layers. The archenteron, *a*, has begun to decrease in size and its roof to thicken. In the mid-dorsal line the notochord cells, *n*, are distinct. As the section was somewhat oblique, it shows the state of affairs in two parts of the egg. On the left (the more posterior), the mesoderm cells, *m*, form a distinct layer of small polygonal cells. The endoderm, *en*, is a single layer of cells whose long axes are, as a whole, directed radially to the archenteron, in contrast to the end-to-end arrangement, previously existing. Near the chorda the two layers fuse with one another and also with the notochord. There is no trace of a cleft leading from the archenteron into the mesoderm, therefore no indication of archenteric pouches. On the right side of the egg, it is difficult to separate endoderm from mesoderm cells, except, perhaps, by their position. In the anterior parts of the embryo, conditions are not very different from that shown in fig. 39, except that the archenteron has become smaller and its roof thicker.



40 41

Figs. 40 and 41

At the next period, fig. 41, the 49th section of 102, the surface views correspond to plate 1, fig. 7. The mesoderm has extended ventrally and is entirely distinct from the other germ layers, except near the notochord, *n*, where it is not yet quite separate. Its rounded cells are arranged, roughly speaking, into two layers. Those of the inner layer are often smaller and somewhat elongated. By joining end-to-end, they give indications of forming a definite layer, the forerunner of the splanchnopleure. The archenteron, *a,* has become very small. The number of cells bounding it is very much less than before. In the mid-dorsal line the row of notochord cells, *n,* begins to arch up.

It is not my purpose to extend the account of the development of the gastral mesoderm, since its origin, by a process of delamination among the cells of the archenteric roof has been clearly shown, although in later stages, the cells of the mesoderm come to look very much like ectoderm. The coelom does not appear until much later.

The decrease in size of the archenteron must be commented upon. Part of this decrease appears to be taken up by the decrease in size of the egg which takes place at this time. The remainder may be taken up by an imbibition of the archenteric fluid by the yolk cells, so that they become larger. This appears to be the case. But aside from the mere decrease in size of the archenteron, a much more important question may be asked. What becomes of the yolk cells bounding the archenteron in the earlier stages? Compare fig. 38 or 40 with 41. Do the cells simply pull away from the edge and become lost in the general mass of yolk cells or do they follow some other course? While it is impossible to decide between these two views, there is some evidence, *i.e.*, the varying shape of the cells which supports the former view. But without denying this view or asserting that the suggestion about to be made is correct, I wish to point out one or two things: First, the cells bounding the dorsal and lateral walls of the archenteron in fig. 41, are as large if not larger than those in the earlier stages. Second, the mesoderm has undergone a considerable extension ventrally without any decrease in thickness and very little decrease in the size of the cells. These facts induce me to believe that some of the floor cells of the archenteron (fig. 41, for example) later come to lie in the lateral walls. The cells of the lateral walls, in turn, go to furnish the material for the extension of the mesoderm. There is no conclusive evidence that such is the case.

This view of the ventral extension of the mesoderm in its essential feature, namely, the transformation of the endoderm cells into mesoderm, leading to the ventral extension of the latter, is not very different from the view put forth by Hertwig in 1883, although I find no evidence of archenteric pouches. There is never any cleft among the cells at the point where they are almost entirely separated from the notochord and endoderm cells in fig. 41. Very soon they separate completely.

The peristomal mesoderm can be distinguished earlier than the gastral. It is already apparent ventrally (fig. 33, *vm*), as the archenteric invagination is nearing completion. It can also be distinguished in cross-sections as polygonal cells in the dorsal lip of the blastopore, although it does not surround the immediate edges of the blastopore until the stages shown in fig. 34. The ventral mesoderm can readily be traced back to the yolk cells lying just beneath the lower corner of the blastocoele, opposite the blastopore. They are carried (or move) along bodily, differentiating as they go until at the close of gastrulation they can be seen as a triangular mass forming most of the ventral side of the blastopore (fig. 34, *vm*, cf. figs. 28 to 33). Later on this mass thins out. Part of its uppermost cells come to lie in the posterior wall of the gut.

Brauer's ('97) results on Hypogeophis show clearly that the origin and formation of the mesoderm in the Gymnophionians is much specialized. It is formed by an invagination of animal cells whose sole destiny is the formation of mesoderm and notochord. In the Amphibians proper, there are considerable differences of opinion as to the source of the mesoderm and the mode of its formation.

Unless one has material other than one's own at hand, it is impossible to deny views based on the study of such material. I have had the opportunity to examine the mesoderm formation in only one other Urodele. In cross-sections of eggs of Amblystoma, conditions quite similar to those shown in figs. 36–41 were found. I conclude, therefore, that the formation of the mesoderm in Amblystoma is the same as in Spelerpes, although the degree of invagination at the dorsal lip has not been ascertained for the former. Brachet's careful work on the Axolotl and frog, King's on Bufo, as well as Adler's work, all force me to the conclusion, that in the more generalized forms, like Triton, Rana, Bufo, etc., the mesoderm cells have arisen by differentiation in situ from the roof cells of the archenteron. In more specialized forms, like Salamandra maculosa and Hypogeophis, where the process has been modified by the presence of large amounts of yolk, I believe that the mesoderm arises by a true invagination of the ectoderm cells between the endoderm and ectoderm.

Notochord

At the stages shown in fig. 36, or sometimes earlier, it may be distinguished in the mid-dorsal line of the archenteron as a mass of cells which is sometimes thicker than the lateral part of the roof. They are usually more compact than the neighboring lateral cells, which often are somewhat separated. It has another distinguishing feature. Sometimes in sectioning, the lateral parts of the roof of the archenteron are pulled away from the ectoderm but the anlage of the notochord is almost never pulled away. A close study of sections show that no actual union exists between it and the ectoderm. Nevertheless, they cohere in some unknown way. Thus, even before morphological differences are present, the anlage of the notochord may be distinguished by other characteristics than mere position, which serve to indicate the future history of these cells.

Such characteristics serve to distinguish the forerunners of the notochord cells until the stage shown in fig. 38, when it has become a single layer of columnar cells. Its further development is completed in a perfectly orthodox manner for Urodeles. It arches up against the ectoderm, its ends separate from the mesoderm and also from the endoderm which closes beneath it. Finally it forms a rod of cells. The time and degree of arching seem to be correlated with the depth of the neural groove, for, if the latter be deep, the notochord is not rounded up as much as when the groove is shallow, thus lending support to Goette's views on the neural groove mentioned above.

Neurenteric canal

I have not found any neurenteric canal in Spelerpes. It may be very transitory and have been overlooked. I have examined carefully only a few sections which cover the period of fusion of the posterior neural folds. The post-anal gut is readily enough found, but it ends blindly against a solid mass of nerve tissue. In one case, the cells of the neural tube for a short distance were somewhat loosely united in the region in which one would expect to find the canal.

The neurenteric canal was overlooked in Triton for some time, but Schanz ('87) succeeded in finding it. The way in which the blastopore closes and the fact that the neural folds fuse in front of the blastopore renders it probable that there is no neurenteric canal in Spelerpes.

PART 2. EXPERIMENTAL

Introduction

The experiments of H. V. Wilson in pricking frog's eggs suggested to the writer that since the eggs of Spelerpes are white, it might be possible to mark them in some way and so follow the movements of the egg material. The season of 1905 was largely spent in the attempt to find suitable methods. A large number of things that might possibly produce a mark upon the egg were tried. At the very end of the season, it was found that the aniline dye, Nile blue sulphate, produced definite spots upon an egg in which the neural folds were forming and moved with them. The following season demonstrated its applicability to the solutions of the problems under consideration, although it makes a less stable mark on the upper hemisphere in cleavage stages than was anticipated. In later stages this difficulty is scarcely apparent. The reason for this became evident when it was found that the stain had a strong affinity for the yolk granules, leaving the protoplasm unstained. This is well shown by marked eggs which have reached the formation of the neural folds. The folds, if they happen to pass through a stained spot, lose much of their stain, while at either side, no loss occurs. Before cell-walls become numerous, the stain slowly spreads from granule to granule for a considerable distance, though sometimes it follows the cleavage furrows. After the egg becomes well divided into cells, this indefinite spreading largely ceases, apparently because the stain does not easily diffuse through the cell walls. The stain does not injure the egg unless an excessive amount be applied. If this is done, the egg, after a time presents a sort of burned spot in the places where the concentration of stain has been too great. Such injured places are relatively permanent, especially when the injury is considerable. As they do not seriously affect the further development, they are often of advantage rather than otherwise, especially when, as in my experiments, they lie in regions not subject to movements of the egg material, thus furnishing a check upon the movements of the stained spots.

A large number of other aniline dyes have been tried. Most were entirely unsuitable. Chrysoidin produces a yellow mark which does not spread, but fades out in about twenty-four hours. It has been used in some of the studies of the movement of the blastopore. Further trials may show some stain lacking the faults of Nile blue.

Method of staining the living egg

A very definite mark can be made upon the egg in the early cleavage stages, if it is first shelled out of its envelopes, but such eggs sooner or later disintegrate. The egg cannot be marked as it lies within its intact envelopes, so the following method was adopted. The egg is placed on a glass slide and the outer jelly is stripped off with needles leaving the inner capsule intact. Surplus water is removed and the inner capsule punctured with a sharp needle, taking care not to injure the egg. This allows the perivitelline fluid to escape. All fluid about the egg is carefully removed with filter paper, and the least possible amount of dry stain is then applied with a needle in the desired position on the jelly. When a sufficient number of places have been marked, the egg is set aside for a few moments, care being taken to see that it does not stay long enough for the jelly to become dried out. Five or six eggs can usually be marked while waiting for the stain to work through the jelly of the first. When this point is reached the egg is carefully floated in a drop of water and removed from the slide to permanent quarters. The membranes soon swell, leaving a stained spot on the egg. Sometimes the egg rotates within its membranes and new marks may be produced on other parts of the egg if sufficient stain remains in the jelly.

Eggs whose peri-vitelline fluid has been removed are usually incapable of rotation for a time. Before its removal, the egg rotates so quickly that the upper hemisphere is almost always uppermost when the fluid is removed. The simplest way to invert the egg in order to mark the lower hemisphere is to use a perfectly dry second slide. The first slide with its egg is inverted over the second and carefully lowered until the egg adheres well to the second. As it is the drier, the egg sticks to it more strongly than to the first, which is easily removed, leaving the inverted egg behind.

Owing to its inability to rotate, care should be taken to orient the egg, animal pole up, after they are placed in permanent quarters. Unless this is done the egg is subjected to the influences of gravity in abnormal directions, which, as is well known, may produce abnormalities.

The attempt to follow the marks into the interior of the egg by means of sections has failed thus far, since the stain dissolves out in alcohol. By breaking open the egg after preservation but before placing it in alcohol a stained spot made on the dorsal lip was found only a short distance within the egg. It is hoped to be able to work out the movement of material within the egg by means of eggs preserved in formalin.

All drawings of marked eggs, except most of those made to determine the relations existing between the plane of first cleavage and the median plane of the embryo, were made with a camera. Since they are projections of a sphere upon a flat surface, it should be borne in mind that a point on the surface 45° from the egg axis for example, comes to lie approximately four-fifths of the distance from centre to circumference of the drawing. Hence, with any movement of egg material the same amount of movement of the blastopore or marks will appear much greater, the greater its distance from the circumference of the drawing. If the blastopore appears only 30° from the equator of the egg, a real movement of 30° will appear much less than if it had appeared 50° below the equator and moved an equal distance.

The relations between the planes of cleavage and the planes of the embryo

The eggs used in these experiments were marked, usually in two places, on the upper hemisphere. A free-hand drawing was made (except series *K*) upon a circle as a basis, to record the relation between the marks and the plane of cleavage. Further records, usually in the form of drawings, were made at intervals showing the changes in shape and size of marks. When the eggs were marked sufficiently far above the equator and the marks were sufficiently small, they often remained nearly constant after the initial spreading had occurred. But such marks were very likely to fade, since they lay in the more protoplasmic parts of the eggs. Marks made near the equator were not very apt to fade but they became involved in the series of changes about the equator described in another place. Hence, even with drawings, it was not always possible to find a single interpretation only, of their movements, from which to derive their original position. If, however, the marks happened to come in certain positions relative to the blastopore or fell in the median plane of the embryo, no misinterpretation of the data was possible. For example, if the marks happened to lie in the future median plane of the embryo, the mark on the blastopore side moved only a short distance directly into the blastopore, while the one on the other side of the egg moved in the same plane across the ventral side of the egg. As the majority of eggs were marked parallel to the first plane of cleavage and as all doubtful cases were discarded, it may help explain why cases of coincidence between the first plane and the median plane of the embryo were more numerous than cases of separation by 90.°[8]

In many cases the first plane of cleavage did not divide the egg equally as has already been noted on page 180. The more extreme cases of such inequality have been noted in the table given below. In other eggs marked before the first plane had extended far, it sometimes happened, when the centre of the stained spot lay directly in the path of the advancing furrow, that it has been deflected from its path, passing around the edge of the stained spot.

In 15 of the successful eggs, the first plane and the median plane of the embryo coincided (table 2, page 214). In 8, they formed an angle of 90° with each other. In 14, still other angles were formed.

Series *E* is notable because in all of the eggs, the median plane of the embryo coincided with the first plane of cleavage. From Series *D* we learn that no necessary connection exists between the plane of the egg which passes through the middle of the blastopore and the median plane of the embryo. This lack of agreement is further emphasized in the studies made upon the movements of the blastopore (vide infra). In some cases the median plane of the embryo did not coincide with the first plane of cleavage, although it lay at one side parallel to it.

Of all of the numerous workers, (viz., Newport '50, Pflüger '83, Roux '83 and '03, Born '84, '93 and '94, Rauber '86, Hertwig '93, Jordan '93, Eycleshymer '95 and '04, Endres '95, Bataillion '96, Schultze '99, Kopsch '00, Spemann '02, Morgan and Boring '03, Brachet '04, and Jenkison '09), who have discussed the relation existing between the first plane of cleavage and the median plane of the embryo in Amphibians, no one has described a series of experiments in which there was an exact coincidence between either the first or second planes of cleavage and the median plane of the embryo. Eycleshymer's ('04) observations on the eggs of Necturus are especially interesting because, although the first plane of cleavage in the lower hemisphere could be fol-

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[8] The angle between the first plane of cleavage and the median plane of the embryo was determined by inspection.

TABLE 2

SERIES	NUMBER OF EGGS MARKED	NUMBER OF EGGS SUCCESSFUL	ANGLE BETWEEN FIRST PLANE OF CLEAVAGE AND MEDIUM PLANE OF EMBRYO, DET. BY:—		REMARKS
			Blastopore	Neural groove	
A	14	1	45		Outer membranes entirely removed
B	15	0	—		
C	6	1	0		
D	11	4	70	90	
			90	45	
			0	0	
			—	0	
E	12	7			Two eggs died after blastopore appeared. In one, the 1st plane divided the egg unequally
			0		
			0		
			0		
			0		
			0		
			9		
			—	0	
F	41	8	0		Vesicle formed in this egg.
			25-45		See fig. 10 which is a sketch of this egg
			45		
			35		
			0		
			45-10		Two interpretations
			90		
			90		Vesicle formed
G	11	6	45		
			90		
			45		
			90		
			90		
			80		
H	12	2	0		1st plane somewhat s-shaped
			80		1st plane curved. Both eggs died after blastopore appeared
I	9	0	—		
J	9	4	0		
			0		
			45		
			80		
K	10	5	50		Records in series K kept by camera drawings
			10		
			90		
			0		
			96		First plane of cleavage unequal

lowed to the time of the appearance of the blastopore, no constant relation existed. In spite of the lack of a coincidence between the first plane and the median plane of the embryo, several authors, expecially Roux for the frog, maintain that a causal relation really exists, but fails to manifest itself owing to the presence of disturbing factors. I shall return to this question a little later.

The results of many observers who found that, in a large percentage of cases, the median plane of the embryo coincided with the first plane of cleavage, point, in a general way, as Roux maintains, to some sort of relation between the two. Nevertheless, I believe with Schultze ('99) that whatever the relation existing, it is equally certain that it is not a causal one. In Spelerpes, it is certain that there is no definite relation existing between either the first or second plane of cleavage and the median plane of the embryo. My results appear to show, furthermore, that as far as Spelerpes is concerned, eggs selected at random have not been subject to any external influences which might make the first plane of cleavage take a different position from what it would have had, had such influences been absent. Roux ('03) has taken the stand that frog's eggs, as obtained from the field, are not normal, that is, they have not been kept from those conditions which cause the first plane of cleavage to deviate from its proper position and therefore, he has objected to the use of results obtained on such eggs. It is well known that in nature frog eggs when first deposited lie with their axes in all sorts of positions relative to gravity. Only after some time do they become free to rotate and thus bring their axes into a vertical position. According to Roux, eggs lying with their axes placed obliquely are in abnormal positions. Thus, paradoxically enough, the eggs although normally laid, are laid in abnormal positions. It has been shown by Born that, when the egg's axis is placed obliquely to gravity, the contents rotate. Roux believes that the entrance point of the sperm determines the first plane of cleavage and the median plane of the embryo. Now, since he fails to find an exact agreement between the two, some explanation is necessary, which is found in the fact that the copulation path of the sperm nucleus is deflected by a rotation of the contents of the egg away from the plane occupied by the penetration path. Since the former determines the direction of the plane of cell division it explains, formally at least, those cases in which the first plane of cleavage deviates from the median plane of the embryo. In Spelerpes, owing to the relative large perivitelline space present when the egg is laid and the long time elapsing before the first division, the egg would seem to be under normal conditions in Roux's sense, from the very start. Hence I believe no objections can be raised on this ground to the use of eggs collected in the field.

The lack of agreement between the first plane of cleavage and the median plane of the embryo has an important bearing on the numerous experiments made to test the potentiality of the blastomeres. If one of the first two blastomeres of the frog be killed, a half embryo may develop from the living one. Morgan ('95) by first killing one of the blastomeres and then inverting the egg, was able, often, to produce a whole instead of a half embryo. Schultze ('94) followed by Wetzel ('96), was able to produce double embryos by inverting the egg after the first cleavage was completed. These results, considered in the light of the failure of the median plane of the embryo to coincide with the first plane of cleavage, merely show that, if a *lateral* half of the material destined to the formation of the embryo, be killed (or if the two halves be separated, as in the recent experiments of McClendon, '09) it is able, under suitable conditions, to rearrange itself to form a whole embryo, but it does not prove that the blastomeres are necessarily either equipotent or totipotent. Spemann ('02), by ligaturing the egg of Triton in one of the first planes of cleavage,[9] finds that while the first two blastomeres may produce two embryos if the first plane of cleavage and the median plane of the embryo coincide, that when these two planes lie at right angles with each other, one blastomere develops into an embryo and the other into a structure comparable with the ventral half of an embryo. Spemann's results prove, I believe, that the first two blastomeres are not equipotent unless there is a coincidence between the first plane and the median plane of the embryo. Furthermore, his results show that the egg is not isotropic, as maintained by Hertwig and others.

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[9] He is confirmed by Hrdlicka's results, though not so stated by Hrdlicka.

The reason for this view may become clearer if we consider the position of the entire embryo and its median plane in relation to the first plane of cleavage. When the last two coincide, the first plane of cleavage will separate the material of the two lateral halves of the embryo. When the first plane of cleavage and the median plane of the embryo lie at right angles to each other, it follows, since the head of the embryo is located somewhere between the upper pole of the egg and the equator, and since the blastopore closes at or near the lower pole, that the embryo develops entirely in one blastomere, excepting, possibly, a little of the tail. In this connection, it must not be overlooked that speaking generally, the material out of which a variable amount of the posterior part of the embryo is formed lies in a half ring around the equator and that the two meridians lying 90° or thereabouts from the point of origin of the blastopore, which indicates presumably the median plane of the embryo, are at the posterior limits of the embryonic material; that is, these meridians where they cross the equator, mark out the tail region of the future embryo. Pricking experiments, notably those of Miss King, show that this material moves nearly along these meridians, towards the point of closure of the blastopore.[10] In Spelerpes, this material moves a little obliquely anteriorly (see page 224). Even if the embryonic material lies higher up in the egg than I have indicated, especially in earlier stages as Morgan maintains, it would not affect this argument since this material is supposed by him to move meridianally to the equator.

If the first plane of cleavage and the median plane of the embryo happen to coincide, as they usually do in the frog's egg, the second plane of cleavage, which according to Roux separates anterior and posterior, will coincide with the point of greatest extension of the lateral lips, *i.e.*, the point where the horns of the crescent lie, at the moment that the blastopore becomes a half circle.

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[10] See also below.

Since the material at this point moves along these meridians to the blastopore, it follows that the second plane of cleavage corresponds at least approximately to the position of the posterior end of the neural folds. Accordingly, then, the second plane of cleavage separates not anterior from posterior, but dorsal from ventral, as Kopsch and Spemann claim. In the four-cell stage then, there are two dorsal and two ventral blastomeres.

If the second plane of cleavage happens to coincide with the median plane of the embryo, then it follows that the first plane separates a dorsal and a ventral blastomere.

If an angle of 45° is made between the two first planes and the median plane of the embryo, one of the first four blastomeres will be wholly dorsal and one ventral, while each of the others will be half dorsal and half ventral. Still other angles will produce correspondingly various degrees of distribution of the egg material into the various blastomeres.

The egg axis, according to the view I have adopted of the position of the embryo on the egg, becomes not the dorso-ventral axis of the embryo but the antero-posterior. Differences existing in the position of the head of the embryo will modify this statement slightly. In other words, the future embryo, in the narrower sense, lies almost entirely in one half of the egg, and before gastrulation mostly above the equator.

Since most of the work done to test the equipotence or totipotence of the egg has, tacitly at least, assumed that two of the blastomeres of the four celled stage were anterior and two posterior, instead of dorsal and ventral, it follows that the living blastomeres in those eggs in which the anterior blastomeres so-called, or a corresponding amount of the material of the egg had been killed, failed to develop beyond the gastrula stage, not because of the injury, but because of an inherent inability to form an embryo. Spemann, in his experiments, found that the ventral blastomere remained alive a long time, but without differentiating. Evidence is not lacking that others have found the same thing. Thus, Hrdlicka describes ventral structures and more recently Morgan and Lacquer describe posterior[11] half-gastrulae. The fact that two of the blastomeres of the four-cell stage are ventral instead of posterior, explains why no one, since Roux first described a possible posterior half embryo, has succeeded in producing such forms. Various explanations of this lack have been offered. Morgan concludes that the "failure of the posterior lip to differentiate further can only be accounted for as due to the absence of the anterior parts." This opinion is at variance with the well-known capacity for self-differentiation of the various parts of the egg. Brachet ('04), reached conclusions similar to my own, although he thinks the absence of the anterior parts has some effect in preventing the appearance of the blastopore. It would be interesting to know whether or not a whole embryo, if any, would develop from the two ventral blastomeres if the egg were inverted after killing the two dorsal blastomeres.

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[11] More correctly, ventral.

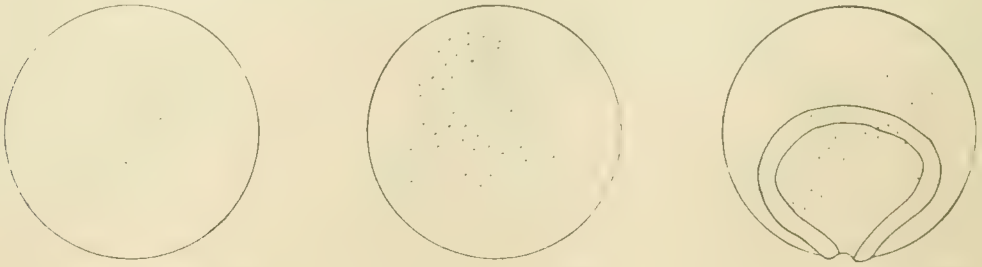
From all of these considerations, I conclude that the amphibian egg is not isotropic but is a mosaic. This mosaic does not reveal itself in its mode of cleavage as happens in many other eggs. The observed power of a single blastomere to produce a whole embryo is dependent, not upon the totipotency of the blastomere, but because it happens to contain the anlagen of all the parts of the egg, which given opportunity, regenerates—to use a rather doubtful term—the missing parts.

Position of the embryo

In 1905 and 1906, records by means of drawings were kept on 41 eggs, marked near the upper pole in early cleavage stages. Sixteen of these gave definite results. The rest either died or the stain faded or spread in such a way that no definite conclusions could be drawn as to the position of the material for the anterior connective in the uncleaved egg, although many showed that the embryo must have developed largely upon the upper hemisphere of the egg. None of them offered evidence that the embryo developed mainly over the lower hemisphere. The eggs marked to determine the relation between the first planes of cleavage and the median plane of the embryo, confirm these results. With two exceptions, which will be commented upon later, the same is true for the eggs marked about the equator. In a few experiments the upper four cells at the eight-cell stage were destroyed. No embryo developed, although some gastrulated.

The embryos which developed in an oblique position on the upper hemisphere in some eggs marked about the equator and described below, appears to be correlated with shiftings of the blastopore, although the data is too scanty to determine this with certainty.

The results from the 16 successful eggs were somewhat variable. The transverse neural folds formed either at the original upper pole of the egg or at some point not over 40° beyond. Part of this variation may be ascribed to difficulties in exactly locating the upper pole. But as the blastopore has been found to move in varying amounts and as the embryo when first formed varies somewhat in length, the differences are partly real.



42 43 44

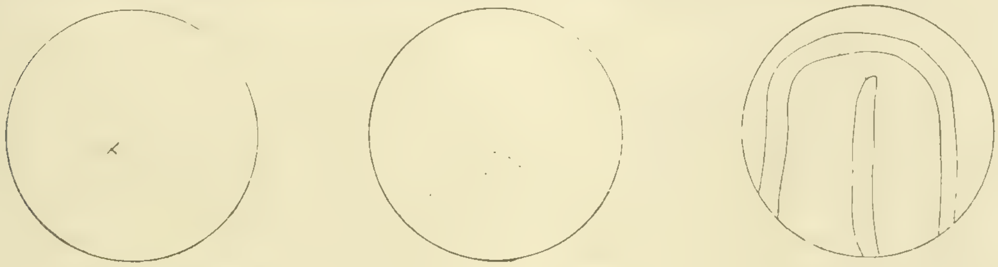
Figs. 42–44

Owing to lack of space only two eggs can receive individual description. In the figures, the stained places are indicated by stippling. The closer the stippling, the stronger the stain. However, the relative differences of stain indicated, are, as a rule, applicable only to the figure to which they belong.

Series *A*. No. 1. Eggs in 12 to 32 celled stage, marked May 9, 8 P.M. First drawing made next morning, 6.45 A.M. Fig. 42 shows the stained areas as a sort of hollow square on the upper hemisphere, with a projection to one side. Three of the corners are more heavily stained. About thirty-four hours later, the stain appeared as shown in fig. 43. As the stain remained nearly constant, no further drawings were made until May 14, 8:30 A.M., when the embryo was well developed (fig. 44). The anterior connective lies in about the middle of the stained area.

Series *B*. Eggs in early cleavage stages, marked May 16, 6:30 P.M. and immediately drawn. No. 4, fig. 45. Four small spots were made upon the upper surface of the egg around the upper pole which is indicated by the cross. It lies a little eccentric. May 18, 12 M. (fig. 46). The stain has spread and now forms a small patch in the region occupied by the original spots. Records made at intervals show no further changes in the stained areas. May 23, 11 A.M., the sketch shown in fig. 47 was made. The stain lies well within the neural folds on the left side. One end crosses the anterior end of the neural groove. The egg has rotated somewhat (see page 191). I conclude that the anterior connective must have been formed some distance beyond the upper pole.

The results obtained from the other eggs while similar, show that as stated above, some variation exists in the location of the anterior connective. Provisionally we may say that the anterior connective develops 25°, on the average, anterior to the upper pole. The advance of the dorsal lip is also variable. Still, I think we shall not be far wrong in assigning, provisionally, a value of 35° to its advance. If, now, it appears 35° below the equator, we find that its median part finally lies 70° below the equator. (The free ends of the blastopore may reach further.) This would give the embryo an extreme length of 185° when it first appears. This figure is not far from correct. I have not measured the embryo, but in preserved material, the anterior connective at its first appearance lies about opposite the blastopore. In a few eggs studied in sections, the embryo appears to be only 160° long, while I have a camera drawing of another, in which the anterior connective at the stage shown in plate 1, fig. 9


45 46 47

Figs. 45–47

is only 130° from the blastopore. The length of the embryo, then, is somewhat variable.

There is still another sort of evidence to show that the anterior connective lies anterior to the upper pole. Its cells have small yolk granules. Only a very small region of ectoderm anterior to the connective has these granules. Now, since the granules maintain their original position until the neural folds begin to develop, it follows that the neural folds lie anterior to the upper pole.

There are two eggs among the ones marked about the equator (see below) in which the embryo apparently formed in greater measure than is usual on the lower hemisphere. The explanation of one of these cases is probably connected with the fact that, after the eggs were marked and placed on the inclined mirror, the primary egg axis failed to become perpendicular.

The case of the other egg is not so clear. There is nothing exceptional about the position of the blastopore, although the marks dorsal to the lip failed to extend into it. But the anterior connective appeared just above the equator. That is, it was a short embryo, not over 130° in length. Two days later, the imperfect embryo lay on top of the egg, but to one side. This embryo is probably comparable with the short one mentioned above. It is interesting to note that a short embryo may form its anterior connective in a position similar to that of the frog.

The series in which both these eggs occurred showed more extensive movements of the blastopore than the other series of this year and moreover, nearly all the embryos lay at one side of the upper hemisphere.

Since the supposition that the embryo of the frog was formed in the upper hemisphere as maintained by earlier workers, was shown by Pflüger and Roux to be incorrect, numerous other workers have attempted to demonstrate the position of the embryo. Their methods have consisted either in preventing the egg from rotating, or in puncturing its surface. The results fail to agree, but I believe this disagreement can be explained in one of two ways, *i.e.*, either it is due to preconceived ideas upon the subject, or more likely to actual differences in the forms used. In general, it has been found that the transverse neural folds of the Urodeles lie nearer the upper pole than those of the Anura. With the exception of Barfurth, who, believing that his own methods had 'grösser Mangel,' locates the head of the Axolotl near the equator, the workers on Urodeles, (viz., Eycleshymer '95 , '04, Jordan '93, Brachet '03), agree in locating the head of the embryo near the upper pole. Schultze ('90) would locate the entire embryo of the Axolotl on the upper hemisphere, but an examination of his figures shows that they can be equally well interpreted in accordance with the view just mentioned. In this same paper, Schultze maintains that the entire embryo of the frog develops on the upper hemisphere and gives excellent data for his position. But Pflüger ('83), Born ('93) and Hertwig ('93) in compressed eggs, observed that the frog's embryo developed on the lower hemisphere, its head lying near the equator. No one, as far as I know, has compressed the eggs of a Urodele and recorded the embryo as appearing on the lower hemisphere. Among the workers who have endeavored to locate the embryo by means of punctures, Eycleshymer ('93 and '04) is the only one who has found the head of Rana as also of Bufo and Acris, appearing at the upper pole. Apparently, there is real variation in the position of the head of the embryo in different species, as has been shown by Morgan ('06), when he found in centrifuged eggs that the head of the toad appeared somewhat higher up than did that of the frog. Morgan and Tsuda ('94), and Wilson ('03) agree in locating the transverse neural folds of the frog slightly above the equator, while King ('01) and Morgan ('06), as already mentioned, find that the embryo of the toad appears nearer the upper pole of the egg. It is interesting to note that, in the eggs of Hypogeophis, as described by Brauer, the embryo must form entirely above the equator, owing to the large mass of yolk present. Ikeda ('02) presents results which are somewhat variable but in general tend to support this view.

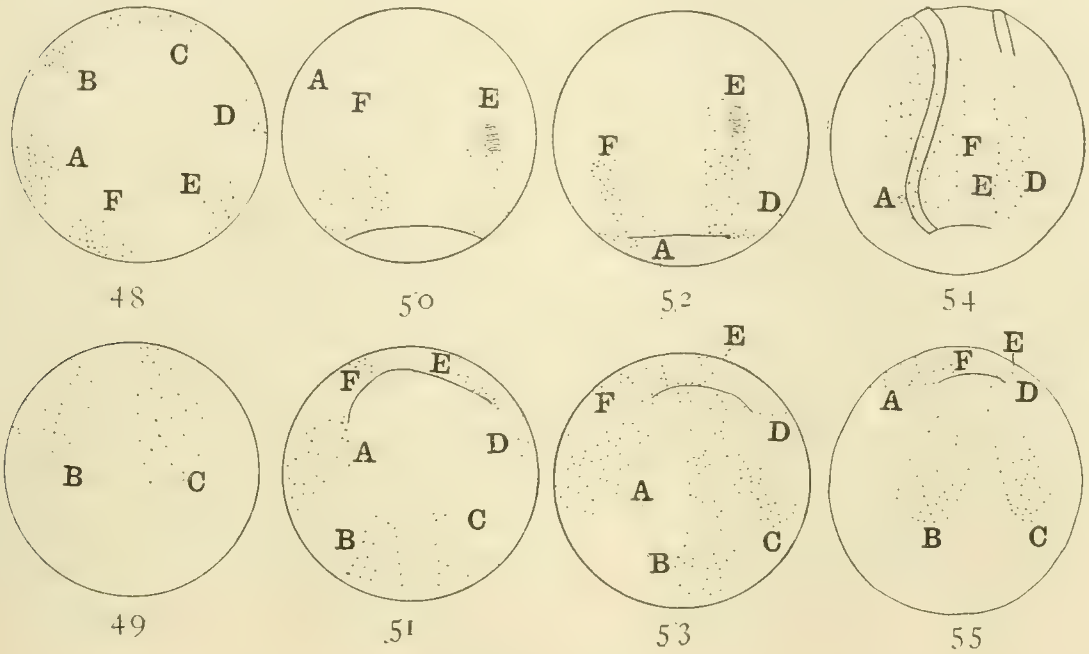
With the data at hand, we are justified in concluding that the amphibian embryo develops almost entirely in a vertical half of the egg, the tail appearing near the lower pole, while the anterior end of the body develops in greater or less degree in the upper hemisphere, depending upon the particular species. The position of the head of the embryo seems correlated with the length of the embryo, so that the longer the embryo, the higher up on the egg it develops. It is interesting to note that the shorter embryos of the frog and toad have a well-developed sense plate. It may be we err in using the anterior connective as the basis of our comparison of the lengths of the embryos. It would seem as though the sense plate were as much a part of the embryo in the narrower sense, as the neural folds themselves, even although they do not function as sensory organs.

Eggs marked about the equator

When eggs are marked about the equator, a few hours before gastrulation begins, with a series of spots, an interesting series of movements of egg-material may be observed. The movement described by Kopsch in the frog's egg are similar in some respects. Bles ('05) has given a figure in which the pigment of a gastrulating egg of Xenopus laevis is arranged in bands similar to the ones produced artificially in the eggs of Spelerpes. I have seen similar natural streaks in the eggs of Amblystoma and one species of frog.

Records were kept by means of drawings on 34 eggs, 25 of which were successful, although not all lived until the embryo appeared. Confirmatory results have also appeared in eggs marked for other purposes.

The history of the marks as they appeared in the egg shown in figs. 48–55 may be taken as a typical case. It was one of a series marked May 9, 8 P.M. The first drawing (fig. 48) was made next morning at ten. The upper hemisphere only is shown since the marks were invisible from beneath. There are six stained spots placed at unequal intervals about the egg, just above the equator. For convenience of reference, the marks have been lettered. The side of a mark nearest the blastopore is arbitrarily called the proximal end, the other, the distal. At 3 P.M., the blastopore had appeared, lying well below *E* and the equator, in the yolk-cells and extending towards *D*. There was no change in the appearance of the stained spots. Next afternoon there was still no change in the marks when viewed from above, but on inverting the egg, a remarkable series of changes had occurred. Fig. 50 is a view of the blastopore side of the egg. The equator is to be thought of as passing near the centre of the drawing. Fig. 49 shows the opposite side, while fig. 51 shows the lower hemisphere only. In figs. 50 and 51, marks *A, D, F,* and *E,* have become elongated bands and extend into the blastopore, while *B* and *C* (figs. 49–51) have elongated toward it. Nearly twenty-four hours later, viz., May 12, 4:30 P.M., (figs. 52 and 53) further changes of like nature have occurred. *E* and *F* are little, if any, longer than on the previous day, although at the blastopore ends, they are closer together (compare fig. 51 with 53). The same lack of elongation is true of *A* and *D* (fig. 53). *A* has swung around so that it now enters the blastopore from the lower side. *F* now enters the blastopore at the corner. The blastopore has undergone considerable changes in shape, which will be discussed in another place. Great changes have taken place in *B* and *C.* They have elongated and now extend into the lower side of the blastopore. As in earlier stages, all bands are conspicuously narrower towards their blastopore end. They are somewhat



Figs. 48–55

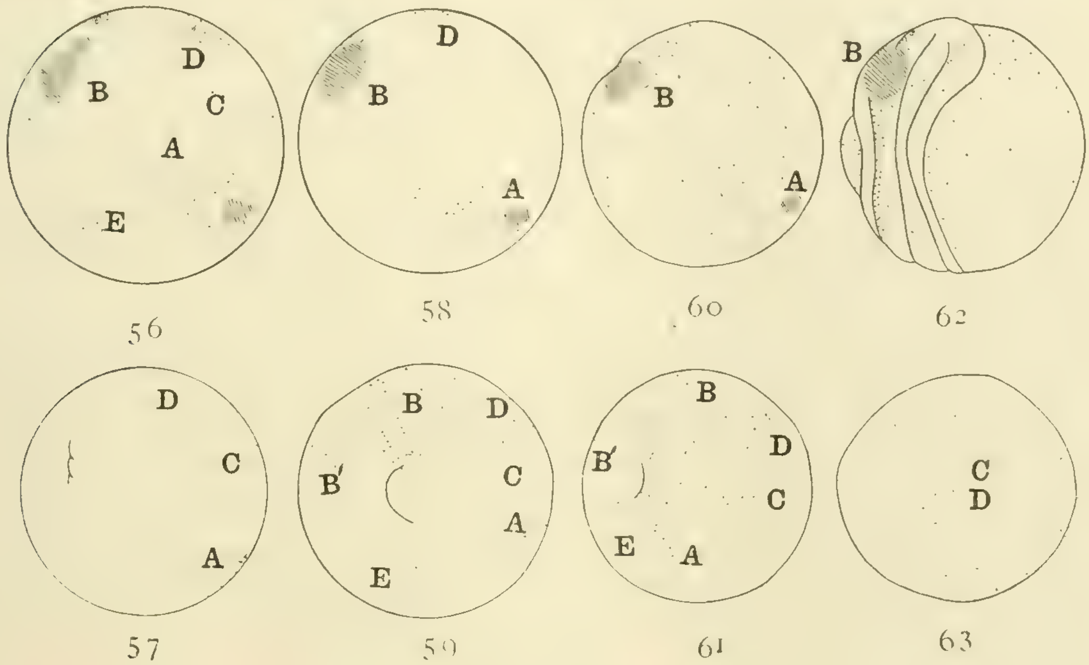
curved. It is noticeable, moreover, that the distal end retains approximately, its original shape and size.

About two days later, May 14, 1:30 P.M. (figs. 54–55), a slightly imperfect embryo had appeared on the upper surface of the egg. Further changes in the marks have taken place. The blastopore has shortened and at the same time *A* and *D* have swung dorsally, coalescing with *F* and *E*, respectively. *B* and *C* have continued the movement blastopore-wards, but now, in part at least, the advance has been one of the band as a whole. The distal end has moved closer to the blastopore, which results in a shortening of the bands. This shortening is correlated with the elongation of the embryo, which has taken place meanwhile. In fig. 55, the anterior connective lies out of sight, just beyond the lower edge of the drawing.

All the eggs marked in this way show similar movements which vary only in details. The lower hemisphere of the eggs of the first series described above were studied by inverting the egg at intervals. To avoid this necessity, the eggs of the other series, illustrated by figs. 56–63 were placed on an inclined mirror in a Stender dish filled with water and the image of the lower hemisphere studied with the microscope. The use of this mirror has one serious objection aside from a certain indistinctness of the image. The mirror reflects only part of the lower hemisphere, the missing part being the equatorial region furtherest from the mirror. At the opposite side of the egg, a little of the upper surface is reflected. Thus, the circumference of the drawing of the lower hemisphere does not represent the real equator of the egg as it does in the drawing of the upper hemisphere. The eggs although unable to rotate freely within their membranes, were free to adjust themselves to gravity, as care was taken to prevent them from becoming attached to the mirror's surface. Consequently, it was assumed that the primary axis would take the perpendicular. Apparently, such was not always the case, to judge from one or two instances, which have been discussed above.

The discrepancies apparent in the figures between the marks on different days, especially in the upper hemisphere, are the results of differences in judgment or of observation as to whether a given mark should be recorded or not. If a mark was faint it might be recorded one day and the next considered too faint to record.

Figs. 56–63 represent the changes observed in an egg, whose lower hemisphere was studied in the way just described. The upper hemisphere is shown in the upper row, the lower in the lower row. The movements of the marks are so clearly shown that a detailed description is not necessary. It should, however, be noted that in the region dorsal to the blastopore, the marks are too far above it to be drawn into it. In figs. 62–63 the stain has faded so badly that it is no longer of much value.



Figs. 56–63

The interpretation of the movements described above is not difficult. As already stated, Nile blue sulphate stains the yolk granules. Hence the extension of the marks denotes cell division followed by an active migration of some of the parts towards the blastopore. The region of this cellular activity lies in a band about the equator. The cell activity may begin above the dorsal lip slightly in advance of that on the opposite side of the egg, since it has been noted sometimes, that marks lying above the dorsal lip begin to extend somewhat before those of the opposite side. Aided by this start, and the nearness of the blastopore, which remains nearly stationary, the cells above the lip reach it first, then the cells of the lateral parts of the equator and finally those from the opposite side of the egg. Judging from the light color of the stain in the proximal ends of the ventral marks, only a comparatively small amount of material from the equatorial region reaches the blastopore, the rest having been distributed along the way. While the daughter cells from the equatorial region are moving blastopore-wards, the cells lying between them and the blastopore are moving in the same direction with the result that nearly all the lower hemisphere becomes invaginated. Cell division appears to be less active among these last, since stains made in this region usually move bodily into the blastopore without extending into bands.

The cells dorsal to the blastopore move into it along their respective meridians. Those lateral to it tend to enter from below, hence their proximal ends swing ventrally, the result being a curved mark. The marks opposite the blastopore move along meridinal lines, generally speaking.

Towards the end of gastrulation, certain changes concerned with the development of the neural plate and dorsal parts of the embryo affect the movements of the lateral marks. When the cells of the neural plate begin to move up, the lateral marks, which lie near the edges of the plate, move with them, thus reversing the direction of their former movement.

The question of concrescence is taken up in another place.

Eggs marked in various places about the blastopore

The typical changes in the shape of the blastopore have been described. In marked eggs, the presence of stained places brings to light certain remarkable shiftings of the blastopore, which are dealt with in the present section.

A brief account of the matter in a few eggs will suffice to give an idea of these movements. My data are not sufficient to determine the exact age at which the process begins, but there is considerable evidence that it does not start until about thirty-six hours after the commencement of gastrulation.

When the observations were made it was supposed that the shape of the blastopore would enable one to determine the comparative age of the egg, but this method has little value. The only sure way is to isolate individual eggs and to observe the first appearance of the blastopore in each egg.

All the eggs described were marked and the movements of the blastopore studied by inverting the eggs at intervals. The secret of using two slides to invert the egg was not known when these observations were made. The lack of this knowledge prevented as prolonged studies on each egg as was desirable, for, in general, much handling and especially inversion of the egg is injurious, although apparently it does not affect the movements of the cells. Moreover, the eggs were usually marked with aurantia, which usually fades out in about twenty-four hours.

Series *B*. No. 4, figs. 64–68;[12] marked at 7:55 A.M. (fig. 64). There was a large spot immediately above the blastopore and a small one about 45° ventral to it. At 11:50 A.M., (fig. 65), the blastopore had extended to a semicircle. The mark above the lip had elongated and apparently narrowed, although this last change might have been due merely to a change in the angle of vision. There was a new mark at the right, extending ventrally from the corner of the blastopore. It arose doubtless from stain left in the jelly. About three hours later, viz., 3:10 P.M. (fig. 66), the blastopore had retracted into a small arc, which apparently is derived entirely from the previous right half, just as though the left half had been erased from fig. 65. Each of the larger marks lay anteriorly above its respective corner of the blastopore. The small ventral mark had continued to approach the blastopore and in the next drawing, at 6:50 P.M. (fig. 67), lay close to it. The other marks did not change to any great extent except that the right one narrowed. The last drawing of this egg (fig. 68) was made the next morning. The blastopore is now bent dorsally at one corner. The ventral mark had disappeared, presumably into the blastopore. Both dorsal marks were close together. Apparently the egg was about ready for the formation of the neural folds.

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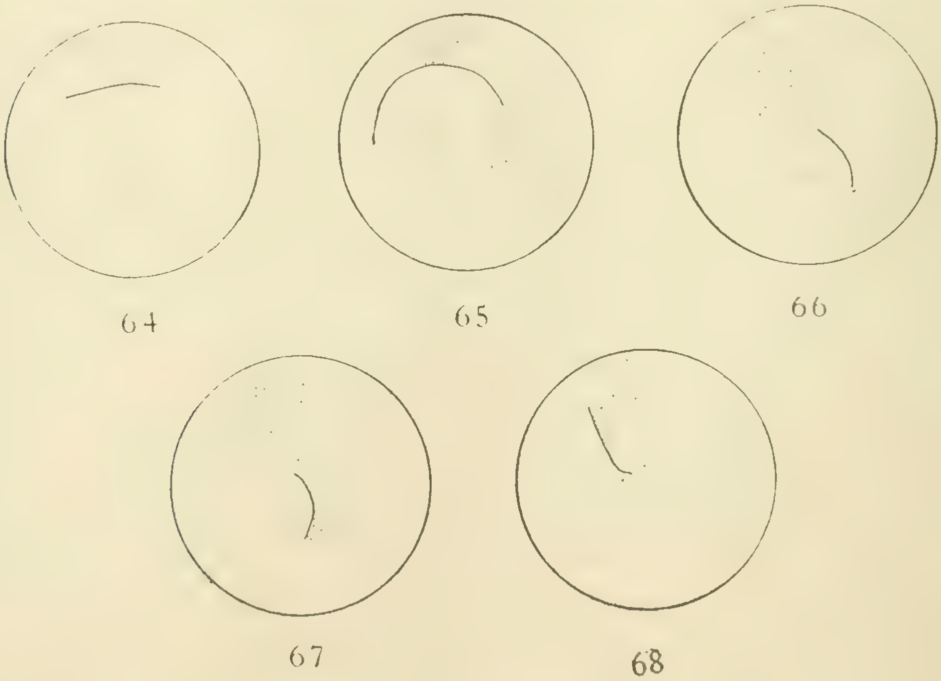
[12] The blastopore is hard to see in the living egg, owing to its lack of pigment. Some of the minor differences recorded in the extent of the blastopore are due to this fact.

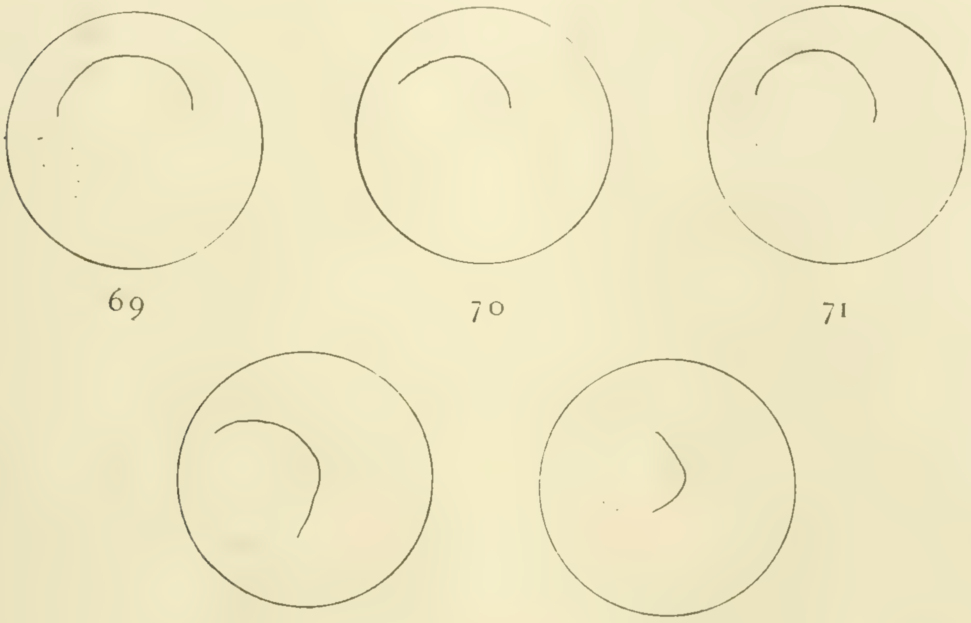
Series *B*. No. 2 (figs. 69–73); marked at 7:40 A.M. (fig. 69). A semi-circular blastopore was present. A stained spot lay above the blastopore a little to the left of the middle. Below the left corner was another, faintly stained. At the next observation, 11:40 A.M., (fig. 70), the blastopore appeared less extensive than before. The mark above the blastopore now extended into it, apparently having elongated in part and in part moved bodily towards it. The lower mark was not as close to its corner as before. 3:05 P.M., (fig. 71) conditions were much the same as at 11:40. 6:45 P.M., (fig. 72), the blastopore had moved clockwise in reference to the marks so that the upper mark now lay at the left corner while the lower lay a little below the right corner. Next morning (fig. 73) the blastopore had shortened, leaving both marks some distance ventral to it.


64 65 66
67 68

Figs. 64–68

Series *B*. No. 1, drawn at 8:00 A.M. (fig. 74). Especial attention is directed to the *A* and *B* marks, the latter a small one which lay close to the blastopore, while the former lay above and a little to the right. 11:40 A.M. (fig. 75) only one-half of the *B* mark was visible. The *A* and *C* marks had spread somewhat. 6:40 P.M. (fig. 76), the *A* mark had extended into a band, reaching the *left* side of the blastopore. The *C* mark appeared drawn out towards the right, showing a movement of the blastopore clock-wise. Next morning (fig. 77) the marks had faded considerably and both *A* and *C* now lay above the blastopore, which had an extension on the right side.

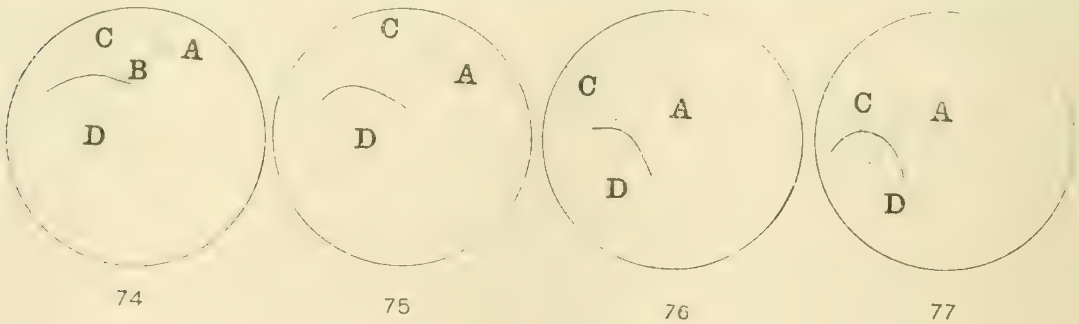
A close study of the eggs marked about the equator show, in many cases, similar movements of the blastopore, especially when only the ends of the marks nearest the blastopore are considered (figs. 48–63).


69 70 71 72 73

Figs. 69–73

It is impossible at present with the meager data at hand, to discuss these movements. All we can say is that they are concerned with the movements of cells into the egg, during the process of gastrulation, and that they are also concerned with the formation of the neural folds. I am inclined to believe that they represent a specialized process of gastrulation, though Eycleshymer notes that the blastopore of Amblystoma does not always extend symmetrically. It is quite evident from my observations that the movements of cells are not equally fast on h sides of the blastopore. I have described the blastopore as shifting, but this was merely a convenient view point. In reality it is due to the shifting of cells relative to the blastopore, i.e., the opening left by infolding cell masses.

There is one apparent correlation between this shifting of the blastopore and sections. Fig. 36, a cross-section of an egg somewhere about this same period of development, shows at the level of the archenteron floor at the corners a mass of loose cells. Similar sections through other eggs at this period often show loose cells on one side only of the egg, (fig. 37, right), the cells on the other side, having already become united. The row of cells lining the archenteron at the corners are usually united into a layer (fig. 36, left side). The separated cells lining the archenteron at the right (fig. 36) are not found as frequently. Now, the forerunners of the ventral mesoderm and the posterior part of the gastral mesoderm dorsal to the blastopore, lay at the equator of the egg before gastrulation began. It is not unreasonable to suppose that the mesoderm cells which are to lie in the vacant places shown in fig. 36, also lay about the equator of the egg. The only other assumption necessary is to suppose that these cells are not invaginated at exactly the same time the rest of the cells are, but that they move somewhat independently of the general process. It is easy to derive a large part of the mesoderm cells from the floor cells of the blastocoele, since they can be seen actively migrating into positions where they must eventually become mesoderm. The yolk-cells of the equatorial region are naturally somewhat less free to pursue an entirely independent course to their final position.



Figs. 74–77

Amount of advance of dorsal lip

An examination of the ventral views of the eggs shown in figs. 48–63, shows that aside from the shiftings of the blastopore, the movement of the dorsal lip ventrally is much less than that of the frog. After their initial elongation, marks above the dorsal lip undergo comparatively little extension until after the embryo begins to lengthen, while those ventral to the blastopore elongate to a large degree. Since the blastopore forms well below the edges of the dorsal marks, it becomes evident that most of the initial movement results from the extension of the marks into the blastopore and not from a movement of the dorsal lip. A comparison of various eggs shows that the amount the lip advances, as nearly as could be determined, varies from 20° to 35°.

During recent years, a fairly general agreement has been reached regarding the amount of movement of the dorsal lip of the blastopore of the frog's egg. Kopsch ('95), by means of photographs of the living egg, found that the dorsal lip formed 25° below the equator and advanced 75° or thereabouts. Wilson ('01), from pricking experiments, reached the same conclusion. Ikeda ('02), working with Rachophorus, found that the dorsal lip appeared 10°–20° below the equator. From pricking experiments, he concluded that the advance was in the neighborhood of 70°. Hence the blastopore closed above the lower pole. In Bufo and Rana, his results were slightly different. Moszkowski ('02) concluded, from some eggs in which an exovate of unknown origin was present, that the dorsal lip appeared 30° below the equator and moved 75°. Miss King ('02), working with Bufo lentiginosus, concluded from eggs studied by Pflüger's method that the blastopore lip advanced 140°. Morgan ('02) states that the amount of the advancement of the dorsal lip is not certain, but he thinks it exceeds 75°. Todd ('04) found, in Rana palustris, that the blastopore closed beyond the lower pole. Assheton ('94) concluded that the advance of the lip was 60° to 70°. Brachet ('03), on morphological grounds admits a lowering of the lip.

Several observers, viz., Lwoff ('94), Eycleshymer ('95) and others, obtained very divergent results within their own experiments. An exovate made in the dorsal lip of the blastopore when it first appeared was sometimes found in the head and at other times in the tail. Miss King's ('02) results explain these discrepancies.[13]

I think, then, it is reasonable to suppose that the advance of the lip is variable, being about 75° for the frog and less in other forms. In Hypogeophis, according to Brauer, its advance is very slight.

The effect of cold and of salt solutions

Before taking up the question of concrescence, I wish to record a few experiments which have considerable bearing on this question and the question of spina bifida embryos.

In the first experiments, the effects of cold were tried on the eggs. This has additional interest, since the eggs of Spelerpes are frequently laid in extremely cold spring water.

Freshly laid eggs were placed in an ice chest with some frog's eggs for comparison. The temperature of the water in which the eggs lay was about 5° C. Beyond a slowing of development —the neural folds appeared twenty-three days after the eggs were placed in the chest—no other effect was apparent. Normal embryos resulted. The frog's eggs, on the other hand, almost all developed abnormally producing various forms of spina bifida.

The effect of salt solutions was also tried. Eggs in early cleavage stages were placed in solutions of NaCl, KCl and LiCl. About 0.5 per cent solutions gave best results. In more concentrated solutions, the eggs died and in weaker ones, no effect was visible. A complete study of these embryos is yet to be made. The most striking feature of the results is the entire absence of ordinary spina bifida embryos. The embryos are somewhat shorter than normal. The posterior ends of the neural folds separate and appear to lie about an uninvaginated yolk mass. These experiments substantiate the results obtained in locating the embryo on the upper hemisphere. In the next section I shall attempt to show why the divergent posterior neural folds cannot substantiate the theory of concrescence.

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[13] See page 236.

Concrescence and convergence

Since His put forth the view of the formation of the animal body by the approximation of two separate lateral halves, much study has been given to determining whether or not this view is true. The tide of opinion has surged first one way and then the other. Patterson ('07), working with chick embryos, has recently demonstrated that, by taking a sufficiently early stage, there is a concrescence of the blastopore lips, which fuse to form the primitive streak. In the Amphibians, after the blastopore has become slit-like, a concrescence by fusion of its lips has been noted. This fusion forms the primitive streak and in this respect is like the chick. I see no objection to this view of concrescence. Another view, maintained by Roux, Hertwig and others, considered that the entire embryo was laid down in two halves in a ring about the equator. They point to spina bifida embryos as proof. Now, there can be little doubt but that much of the material out of which the embryo of many Anura is formed, lies in the equatorial regions at one stage of development. It by no means follows, however, that the two halves of the embryo gradually approach each other, beginning at the head, and fuse, leaving a visible sign of this fusion in the neural groove, as Hertwig and others maintain. I shall return to this.

Morgan ('94, '97, '03), and others, while not admitting concrescence by 'apposition,' believe that part of the embryo, at any rate, is formed by "concrescence from before backward at the dorsal lip of the blastopore," that is, the material to form the dorsal lip and embryo lies in the lateral lips of the blastopore and moves along the parallels of the egg towards the median line, there to fuse, thus causing the dorsal lip to appear to advance. Its material once in place, remains stationary.

Other observers deny any form of concrescence. One group, of whom Jordan ('93) appears to have been the first, followed by Eycleshymer ('95) and Kopsch ('95), holds that the blastopore is formed by convergence. By this they mean that part at least of the material which lay about the egg in a ring moves towards a central point, the lower pole, along the meridians of the egg, that is, at right angles to the direction described by the two groups of authors mentioned above. As a consequence of this movement it must become spread out over the lower hemisphere of the egg. Out of what may be called the anterior half of the ring, more or less (according to the species), the posterior part of the embryo develops.

There is still another group of observers, e. g., Assheton, who believe that the posterior part of the embryo is formed by overgrowth. I understand by this term that the material out of which the posterior part of the embryo is formed, lies in the dorsal lip of the blastopore, and as the lip moves downwards, it spreads out into a plate. I judge, that, according to this view, the lateral edges of the posterior part of the neural region, move downward in lines roughly parallel to each other.

Jordan appears to have reached his conclusions from general observations. Kopsch's brilliant work was the first experimental proof adduced in its support. My own work confirms Kopsch's results, although owing to the specialized mode of blastopore closure in Spelerpes, they are slightly modified. If there were any concrescence, even of the posterior parts of the embryo, the marks should have moved approximately at right angles to the course they actually took. The numerous pricking experiments that have been made on the blastopore of the frog support the view of convergence in a remarkable way, although they have commonly been interpreted in support, in some form or other, of the concrescence theory. Miss King ('02) has made an extended series of these experiments. If a slight exovate be made near the edge of the blastopore in any section, it is later found at the posterior end of the embryo. If the exovate is made a little further above the edge it remains stationary. If, instead of a puncture, a mark had been made with Nile blue sulphate, I should expect that the distal end would remain stationary and the proximal end would finally be found at the posterior end of the embryo, the mark meanwhile having extended into a band. A series of marks made about the equator before gastrulation would, at the close of gastrulation, be found in bands marking out meridians on the lower surface of the egg.[14] If there were any concrescence, one would expect that the exovates made by Miss King in the dorsal lateral lip would have moved into the same meridian as those made at the middle of the dorsal lip. Having reached the median line, they should then have remained stationary, if Morgan's view of the formation of the embryos were correct. They not only do not move into the median line, but an exovate made in the middle of the dorsal lip of the blastopore, where the material is supposed to be coming up from the lateral lips and fusing, actually moves down to the posterior end of the embryo. The actual movements of the exovates completely substantiate the view of the formation of the posterior part of the embryo by convergence.

The view of embryo formation by concrescence agrees with the view of formation by convergence in one essential point, viz.: The material out of which the embryo, or at least its posterior part, is to form lies in a half ring about the equator. The middle of this half ring corresponds to some point in the median line of the embryo. Here the two views part company. According to the former the lateral halves of the embryo lie along this region and join by a movement transverse to the long axis of the embryo. According to convergence, the material out of which the embryo is to form, lies in this same region, but comes together along lines oblique to the long axis of the embryo. But more fundamental yet are the differences between the two views regarding the constitution of the material of the equatorial ring. According to the theory of concrescence—in the frog, to take a specific instance—the material for the head lies just above the dorsal lip. As we pass back along the ring, we come successively to material for the neck, then the sides of the body and finally reach the material for the tail. All we have to do is to swing the two halves together by the tail ends and the embryo is formed. Even the seam, along which the two halves unite, is there, i.e., the neural groove.

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[14] More recent experiments on the eggs of the frog and of Amblystoma confirm these statements.

According to the theory of convergence, the process is more complex. The material out of which the embryo is to form is not yet differentiated, or if differentiated, is still incompletely separated. The material for the tail must be spread out along the entire equatorial band, for example. This follows from the fact that a mark made in the dorsal lip and one in the lateral lip, both reach the tail. The material for the neck region in the frog is not so widely scattered but lies close to the point of origin of the blastopore. As a consequence, it follows that the most complex (or least differentiated) part of the blastopore lip is the dorsal, while the simplest part is 90° from the median line of the embryo, since this part will form only tail and ventral ectoderm. I have not gone into the part played by the ventral lip since it is obvious from my point of view that it furnishes only ventral ectoderm. I am, moreover, speaking of the superficial parts only. I cannot agree, then, with Moszkowski, ('02), who maintains that the formation of the blastopore and the differentiation of the embryo are independent.

Before leaving this question, I wish to point out that spina bifida embryos and their extreme form, ring embryos, can be explained on this view as well as the view of concrescence. The material is already laid down in a ring about the equator. Anything that prevents the movement of this material, as happens when the yolk is prevented from invaginating, forces it to differentiate in situ. In any case, half structures will be formed around the yolk plug, since only half of the material is already present. The experiments of Roux and others in killing one of the first two blastomeres, show, that as long as the material for one-half the embryo is forced to keep its primary arrangement as a half-structure, only half an embryo will develop. It is only when the half is permitted to rearrange itself, as Morgan showed by inverting an egg after killing one blastopore, that a whole embryo can develop (compare also, MacClendon '09). Hence we should not expect that an egg forced to form a spina bifida embryo would form more than a half structure on one side. In these spina bifida embryos, it is interesting to note that the material differentiates a little differently than in the normal embryo. That is, material which, in a normal egg, might form part of the tail, in these embryos, differentiates into the parts in their consecutive order from head to tail. This is to be expected, since Morgan ('02) has already pointed out that ring embryos may be derived from material which ordinarily would not form the embryo. Since the urodelian embryo forms in greater proportion above the equator than does that of most Anura, it follows that the material of the dorsal lip in the former is less complex than that of the latter. The normal dorsal lip of the frog contributes material to all parts of the embryo from the neck to the tail. That of Spelerpes contributes material to only a small part of the posterior end of the embryo.

SUMMARY

1. The uncleaved egg shows three zones of yolk granules, an upper of small ones, a second of larger size, while the lowest contains the largest granules. The anterior part of the embryo develops from the small granules. From the second zone come the posterior part of the embryo in the narrow sense and the posterior part of the archenteric roof besides the ventral ectoderm. The zone of large granules furnish the rest of the embryo.
2. Cleavage is total, unequal and often irregular. After the fourth division, it is confined to the upper hemisphere for a long time.
3. Sometimes the third set of furrows change during their formation from a horizontal to a vertical plane.
4. Sections of eggs in cleavage stages show first, a cap of small cells, then a layer of larger yolk cells and beneath this, forming the greater part of the egg, is the yolk mass, scarcely divided at all into blastomeres.
5. The blastomeres often remain connected for a long time.
6. The egg increases in size up to the time of gastrulation. After the embryo begins to appear, it decreases slightly.

7. The blastopore never extends beyond a half circle. There is no ventral lip and consequently no yolk plug. The blastopore closes by shortening and becomes the anus.

8. No neurenteric canal was found.

9. The neural folds form and close much as in Triton.

10. The neural groove is not the seam of closure of the blastopore lips, neither is it entirely the result of inequalities in rate of growth, nor is it the primitive streak. Its significance appears wholly unknown.

11. Gastrulation takes place by active invagination of the yolk cells on both sides of the blastopore. The amount of invagination is greatest below the lip. Pari passu with this invagination, the yolk cells of the Randzone spread over the ventral surface of the egg.

12. The roof of the archenteron is derived exclusively from yolk (vegetative) cells. Its posterior part is from yolk cells which lay above the lip of the blastopore and which have been invaginated. It is not clear whether the remainder of the roof has arisen from yolk cells which previously formed the floor of the blastocoele, or whether they have arisen from the outer yolk cells by invagination.

13. The gastral mesoderm arises by delamination in situ from the yolk cells forming the roof of the archenteron. The peristomial mesoderm likewise arises from the yolk cells, which in part lay at the lower corners of the blastocoele.

14. The notochord differentiates from the yolk cells lying in the dorsal midline of the archenteron roof.

15. Blue spots may be produced upon the living egg by means of Nile blue sulphate. These spots enable a determination to be made of the relation of various parts of the egg to the future embryo.

16. No constant relation was found between the planes of cleavage and the planes of the embryo.

17. The head of the embryo develops near the upper pole of the egg. Its tail appears somewhat above the lower pole. The embryo is usually 180° long when it first appears.

18. The primary egg axis becomes the anterior posterior axis of the embryo.

19. The amphibian egg may be divided by a vertical plane into halves, one of which is dorsal and the other ventral. This statement is true for the surface of the egg, although the process of gastrulation, may render it less accurate if applied to the interior of the egg. This explains the observed absence of posterior half embryos.

20. Marks made at the equator of the egg spread into bands over the ventral half of the egg during gastrulation. These bands are approximately meridional. They converge at the blastopore.

21. The dorsal lip of Spelerpes is often nearly stationary, although it may advance as much as 35°.

22. The blastopore often shifts to one side or the other of its original position.

23. A temperature of 5° C. has no effect on the eggs of Spelerpes beyond slowing the rate of development.

24. Salt solutions fail to produce more than one form of spina bifida larvae, namely those with divergent posterior neural folds. The anterior two-thirds of the embryo is always intact.

25. The photographic studies of Kopsch, the pricking experiments of King and others and my own work on Spelerpes show that the posterior part (in various amounts, according to the species) of the amphibian embryo is formed by the convergent distribution of the embryonic material, which as gastrulation begins lies in a half ring about the equator, over the lower hemisphere of the egg. There is no concrescence except in a very limited region about the blastopore, after it has become slit-like.

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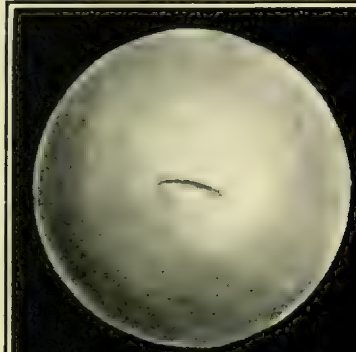
PLATE

EXPLANATION OF FIGURES

1 First indication of cleavage.
2 First furrow; from above.
3 First furrow, more advanced; from below.
4 Second cleavage; from above.
5 Eight-celled stage; from above.
6 More advanced stage of cleavage; from above.
7–14 Formation and closure of neural folds; 7, 9, 11, 13, 14, anterior and dorsal views; 8, 10, 12, posterior views.
15 Two small clutches of eggs, about $\frac{1}{3}$ natural size.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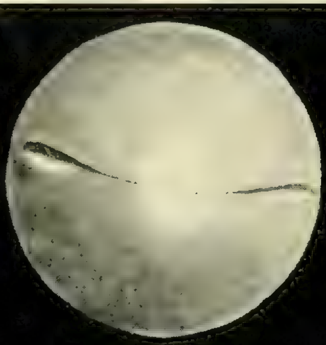
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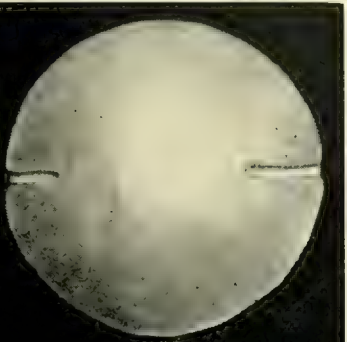
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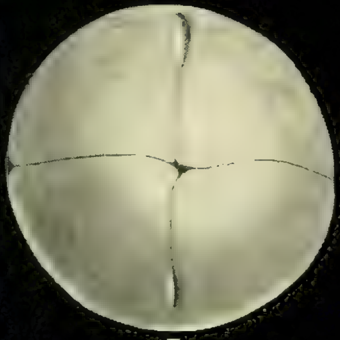
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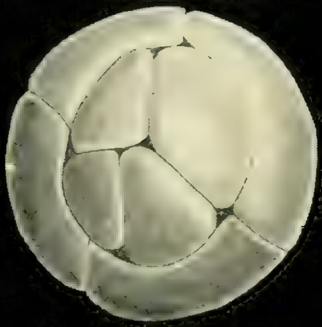
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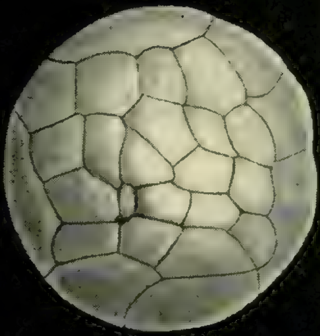
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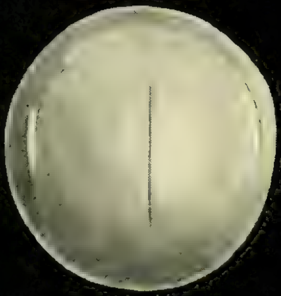
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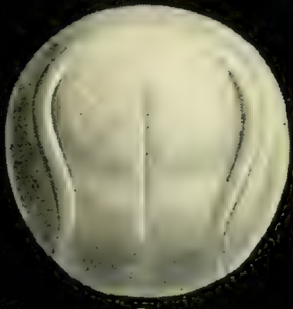
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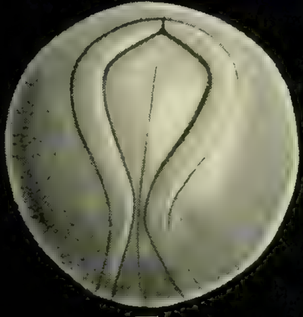
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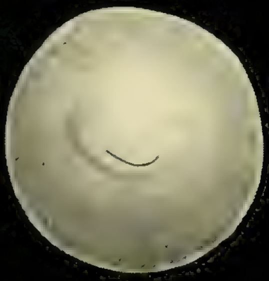
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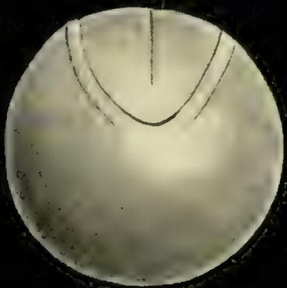
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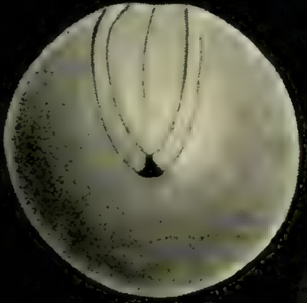
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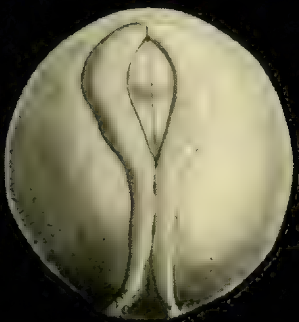
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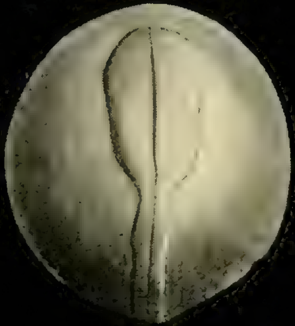
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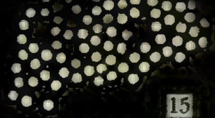
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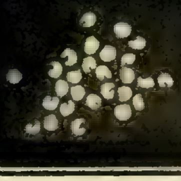

Goodale, del.

THE HISTOGENESIS OF THE PINEAL BODY OF THE SHEEP

H. E. JORDAN

From the Anatomical Laboratory of the University of Virginia

ELEVEN FIGURES

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INTRODUCTION

This investigation is the outcome of a search for histologic evidence indicating a physiologic function for the pineal organ of mammals. In this search a variety of animals were examined, including cat, dog, guinea-pig, rabbit, rat, opossum, calf, sheep and man. In general the macroscopic findings in corpora pinealia of adult mammals seem to negative the presumption of functional importance. In the first five animals above mentioned the body is very small; and the observation that in very large dogs, for instance, it is no larger than in small dogs or that in horses it is

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no larger than in dogs, renders it very improbable that it is an indispensable or even serviceable organ for mammals. Moreover, the further observation that it frequently becomes cystic without producing noticeable morbid effects increases the improbability. Thus in two out of eleven cats examined it consisted simply of a pedunculated vesicle with thin fibro-cellular wall. Its vestigial and variable character in the opossum (Jordan, '11) supplies additional support to the opinion that the pineal body is a rudimentary organ functionally *nil*. The recent work of Exner and Boese ('10) with rabbits is also in accord with this position. These investigators removed the epiphyses of ninety-five young rabbits but were unable to detect any effect on growth or the appearance of sexual maturity among the six that survived. However, in man the pineal body acquires considerable size (ca. 7 mm. x 5 mm.) and exhibits glandular characteristics, suggesting the function of elaborating an internal secretion. Moreover, it frequently becomes the seat of neoplasms whose presence involves certain symptoms which cannot be accounted for on the basis simply of pressure on adjacent structures (Lord, '99; Exner and Boese, '10; and Pappenheimer, '10).

When it was discovered that the sheep's pineal body was of considerable size (ca. 5 mm. x 3 mm. to 8 mm. x 5 mm.) the investigation was restricted to an intensive study of the 'gland' in this form. This seemed the wiser since quantities of material could be obtained for experimentation and preservation in perfectly fresh condition. The large size and the constancy of size (commonly about 7 mm. x 5 mm.; more than fifty bodies were examined) and form, indicated that here, if anywhere, the body should have physiologic significance. Accordingly, the effects of aqueous extracts on various animals were studied,—with the result already recorded (i.e., fall in blood pressure and transient diuresis—Eyster and Jordan, '11). A further step involves the extirpation of the body in the sheep, a work now in progress but as yet without results.

Still another step in the complete study contemplated, was to trace the developmental history of this structure. The present paper is an attempt to record this history from about the second week (2.5 cm.) when the pineal body is merely a shallow ependymal pocket evaginated from the roof of the diencephalon, to the third year when it shows numerous marks of degenerative changes. Assuming, on gross anatomical and experimental basis, that the pineal body of the sheep is a gland, and that it elaborates an internal secretion which exerts specific effects, (probably not indispensable to the life or health of the animal), the primary object was to discover signs of cytoplasmic activity at some period of its development, expressed in granules, vacuoles, etc., indicating secretory function.

MATERIAL AND METHODS

Through the kindness of Dr. Kingsbury, specimens of young sheep embryos, including 2.5 cm. stages, were examined in the embryological collection of Cornell University. My own material includes glands from foetuses of the following lengths: 5, 10, 11 (ca. 1 month), 15, 17, and 21 (ca. $2\frac{1}{2}$ months) cms.; also three heads of foetuses of the last week of gestation (i.e., ca. $4\frac{3}{4}$ months). All of this material was preserved in 10 per cent formaldehyde, and stained in Delafield's or iron-haematoxylin with a variety of counterstains. Besides this foetal material, my sections comprise numerous glands of lambs (ca. 8 months) and adult sheep (up to 3 years). The latter material was fixed in a variety of fluids suited severally to a variety of stains, and sectioned either in paraffin or celloidin. Among the various combinations employed, it seems important to name the following: strong Flemming's fluid (for lipoid granules, and cytoplasmic structure), Helly's fluid (for phaeochrome granules), Müller's and Ehlicki's fluids (for the Weigert-Pal staining technic), Carnoy's fluid, picroacetic, 95 per cent alcohol, and 10 per cent formaldehyde (for general cytoplasmic structure). Iron-haematoxylin was used as a neuroglia stain. For study of the connective tissue elements, Van Gieson's, Mallory's, and Weigert's resorcin fuchsin stains were employed. Non-medullated nerve-fibers were searched for in fresh (from extirpated glands) tissue treated with the methylene blue technic. Macerated material was also employed for various purposes.

The apparently unfortunate gap in the material between full term and about eight months post-natal life leaves no serious lacuna in the developmental history. The gland at term is in all respects, save size, like the gland of the young animal. The sole change is one of increase in bulk (ca. 5 diameters) due to continued proliferation of the parenchymal cells and increase in the amount of connective tissue and neuroglia framework.

The order of description follows the order of development. Regarding stages prior to the 5 cm. phase, it may suffice to note that the epiphysis is simply a shallow pocket lined with proliferating ependymal cells more numerous apically.

THE 5 CM. STAGE OF DEVELOPMENT

At this stage the pineal pocket has taken a slight backward direction. It communicates with the third ventricle through a wide mouth, the pineal recess. It is invested by a very delicate sheath of pia mater. The cells are more crowded around the lumen and in the ventral wall, and here more closely resemble the original ependymal ancestors. Distally and in the dorsal wall the cells have become irregularly stellate or multipolar for the most part, and form a comparatively loose-meshed reticulum (fig. 1). Very delicate fibrils are present, and about three cells in every section contain small melanic granules. A number of cells, including some with the melanic granules, are in mitosis. Transition stages are abundant between true ependymal cells and the differentiating stellate or neuroglia cells. The nuclei of the latter are vesicular, with plasmosome and numerous karyosomes. The cytoplasm is reticular. In the pineal body of this stage occur the first steps in the differentiation process of ependyma into neuroglia. No neuroblasts are recognizable. The neuroglia fibers appear to arise from the spongioplasm by a process of thickening and fusion.

THE 10 CM. STAGE OF DEVELOPMENT

The significant difference between the pineal body of this and earlier stages is a slightly greater backward elongation, coincident with a thickening of the wall and obliteration of the lumen in the terminal portion. The cells of this solid portion are mainly of the irregularly polygonal and stellate type and form a loose-meshed syncytial network with delicate fibrils in all respects similar to that of the 5 cm. stage. Considerably more of the cells contain the melanic granules and a number of all types are in mitosis. The vesicular nuclei contain numerous karyosomes. This stage marks also the beginning of the pial extensions into the substance of the body forming trabeculae (ca. two per section) carrying delicate blood-vessels (fig. 2). Fig. 3 illustrates transition stages between ependyma and neuroglia cells.

THE 11 CM. STAGE OF DEVELOPMENT

At this stage the pineal body has acquired the shape of a thick, rounded, cellular knob with shallow central recess. The walls of the latter contain anteriorly the now well-developed habenular or superior commissure. The parenchyma is more loosely arranged peripherally. The cells are of the same types as above described, more and less developed neuroglia cells. Slightly more contain melanic granules, and in somewhat larger amounts. Fig. 4 *b* illustrates the various types of these granules. Many cells are dividing mitotically. No amitosis can be detected. More pial trabeculae enter the body (ca. three per median section), dividing it peripherally into lobules (four per section). Fig. 4 *a* illustrates the early stage in the differentiation of the inter-neuroglia cells.

THE 15 CM. STAGE OF DEVELOPMENT

This stage is characterized by slightly larger size, and considerably larger amount of melanic cytoplasmic granules, more abundant in the peripheral cells; but more particularly by the appearance of a new feature; several short blind alveoli or cysts situated in the region approximately midway between periphery and center. From four to six alveoli appear in a section. They extend through from eight to ten sections of ten microns, and seem not to branch. Nowhere can they be traced either into the recess or onto the surface. The lumen is spun across with a wide-meshed network of very delicate fibrils (fig. 7) probably a coagulation product. The cells surrounding the lumen are of the usual type, irregularly columnar, non-ciliated and full of granules. The structure appears to be due to the accumulation of a secretion product which exerts pressure peripherally and compels the arrangement of the adjacent cells in a manner to simulate an alveolus. Structures appear at this stage which suggest the interpretation of an invagination of such an alveolus by a pial trabecula with capillaries, forming a sort of glomerulus (fig. 5). Numerous transition stages appear between merely large intercellular vacuoles and the alveoli or cysts.

THE 17 CM. STAGE OF DEVELOPMENT

Nothing essentially new appears at this stage. The pineal body is considerably larger, the pial trabeculae are more numerous, longer and coarser; antero-dorsally the lobulation is distinctly marked on the surface and the amount of the melanic granules has increased, especially in the superficial cells. Moreover, the body is now ramified by connective tissue septa carrying delicate blood-vessels, continuous with the pial trabeculae. These numerous imperfect septa divide the parenchyma into follicular masses. Alveoli of varying calibre and length still remain. There are also more numerous clearly defined parenchymal masses or follicles, surrounding vascular trabeculae. No distinct ciliation can be discerned on the cells lining the alveoli. However the cells lining the pineal recess are ciliated.

THE 21 CM. STAGE OF DEVELOPMENT

The pineal body of this stage of development (ca. $2\frac{1}{2}$ months, i.e., half of gestation period) has attained approximately fourfold the bulk of the 15 cm. stage. It marks the period of the greatest abundance of alveoli and melanic granules (fig. 6). The latter are most abundant in the cells forming the walls of the alveoli. The body appears distinctly black macroscopically at this period. Later it has a dark grayish appearance. At full term its color is as during the earlier weeks, white. The lumina are spanned by delicate strands of a coagulum (fig. 7). The alveoli are more numerous peripherally (fig. 6). They are circular in transverse section, vary considerable in calibre, but have in no case been seen to branch. As regards trabeculae, septa, follicles and cell-types, the body at this stage is in all respects like the former.

THE PINEAL BODY AT TERM

The specimens in hand lack about a week of full term. However, the pineal bodies are in all respects, except size and number of granules, like those of lambs (ca. 8 months). They contain numerous alveoli of varying caliber and length, and considerable of the cells contain small amounts of melanic granules. The characteristic new feature here is the great abundance of follicular arrangements of cells about central vascular connective tissue trabeculae. Since all of the features of the adult pineal gland are here present and in less complicated form, it seems desirable to describe them more in detail.

The body simulates a lobulated gland. The lobules are delimited more or less distinctly by coarser and finer reticular septa of vascular connective tissue continuous throughout, and peripherally, through the coarser trabeculae, with the pia mater. The lobules consist of one or several follicular aggregations of cells. These are spherical or oval masses, the parenchyma of which consist of two distinct types of cells with several intermediate types. The framework of the parenchyma is a reticular structure of delicate neuroglia fibers for the most part continuous with the irregularly polygonal and flattened stellate neuroglia cells. Many of the coarser fibers seem entirely free from the cells, as in typical neuroglia tissue (Huber, '01, '03). The second main type of cell, oval and spheroidal or polyhedral, occupies the interstices of the neuroglia meshwork (fig. 8). Many of the cells still contain numerous melanic granules, more especially peripherally and in the walls of the alveoli or cysts. The latter are still abundant and quite large.

A conspicuous feature is the great vascularity of the pineal body. Frequently capillaries terminate in the form of tangled loops or 'glomeruli' within spaces surrounded by more compact parenchyma. Such spaces in some cases represent alveoli into which a trabecula has carried blood-vessels (fig. 5). It remains to note that both the parenchyma and neuroglia fibers are more abundant peripherally. The latter also are coarser in this region. The entire body, exclusive of the vascular pial trabeculae and a few white nerve fibers, is composed of more highly differentiated or neuroglia cells with fibers, and less highly differentiated or inter-neuroglia cells of the original ependyma of the third ventricle.

THE PINEAL BODY OF LAMBS

In young sheep (ca. 8 months) the pineal body attains the greatest size (ca. 8 mm. x 5 mm.). Its structure is identical with that of bodies at birth; the only difference being one of greater mass of the same constituent elements in the latter (fig. 8). The cell increase has been at least predominantly by mitosis. Occasionally cells are binucleate, the result apparently of amitotic nuclear division, indicating the probability of a second contributory method of multiplication. Compared with the epiphysis of embryos, the nuclei of the constituent cells in young sheep are considerably larger (compare figs. 3 and 8). In the latter, the nucleus measures approximately eight microns and the cell body of the spherical and polygonal type approximately 15 microns. Increase in total size of the pineal body then may be due in part also to the enlargement of individual cells. The greatest increase in bulk (approximately five-fold) during the first year indicates that if the pineal body has a specific function this is most active in the young; and the suggestion frequently made that the body is a gland which elaborates an internal secretion which has to do with the normal growth or the appearance of sexual maturity (sheep mate at from 6 to 8 months) receives support from anatomical facts.

Observations on human corpora pinealia in my collection point to the same conclusion. In an infant of five days the body has the shape and about the size of a small grain of wheat. It is sharply pointed distally and flattened dorso-ventrally. Its dimensions are approximately 3 mm. x 2 mm. x 1 mm. In infants of about a year the body is approximately spherical with a diameter of about 3 mm. In youth the body is largest (ca. 7 mm. x 5 mm.). The pineal bodies of old individuals are considerably smaller.

The presence of so great an amount of melanic granules as to discolor the entire body at about half term, indicates that the secretory activity of the 'gland' may be greatest at this stage of development. But the granules are only relatively more abundant at this stage, being elaborated more abundantly by individual cells. Subsequently the protoplasmic energy seems directed towards cell proliferation. In consequence, the total amount of granules is distributed among a larger munber of cells, each with fewer granules. The entire bulk, however, has remained about the same or possibly slightly increased by small additions, to the time of birth. Thereafter the granules seem actually to disappear in great part.

The iodine test for glycogen was negative. Nor could chromaffine granules be detected. The pineal body can therefore not be regarded as a portion of the so-called 'chromaffine system' nor as ganglionic in character. The histologic features of the pineal body correspond more closely to those of a gland than to any other structure. Its great vascularity, blind acini (cysts), and cytoplasmic granules mark it as a ductless gland.

Weigert-Pal preparations reveal the presence of a bundle of medullated nerve fibers in the proximo-ventral portion of the gland, continuous with the fibers of the posterior commissure. A still more delicate bundle passes from the superior commissure into the proximo-dorsal portion. This may represent the so-called 'pineal nerve.' The final terminations could not be determined. Both in the basal portion and in connection with the blood-vessels occasional delicate fibers could be identified. Methylene blue preparations of fresh tissue (from extirpation experiments) and Cajal and Golgi preparations reveal the presence of still more delicate non-medullated fibers. It could not be determined whether the latter are simply the naked axis-cylinder terminations of the former. Nor can I elucidate the matter of nerve terminations in the parenchyma of the pineal gland. The striking fact that these preparations show, however, is the apparent scarcity of nerve fibers beyond the basal portion. A few alveoli contain small concretions of brain-sand.

In none of my specimens have I been able to discover striated muscle fibers, such as first Nicholas ('00) and later Dimitrova ('01) described for the pineal bodies of Bos taurus. Nor do the trabeculae here contain smooth muscle fibers as noted by some investigators for certan forms (e.g., ox; Illing, '10). Nor have I been able to find any evidence of nerve cells which have been reported in certain mammals by several workers (e.g., Hagemann, '71). Moreover, Flesch's statement that pigment clumps are abundant in the pineal bodies of sheep applies only to those of old individuals; and Dimitrova's observations that no brain-sand occurs in the pineal body of sheep is negatived by findings in my preparations where sabulous concretions appear in small amount in the proximal portion of the pineal bodies of yearling sheep, and in large numbers in various forms in old sheep.

THE PINEAL BODY OF YEARLING SHEEP

After the first year the pineal gland undergoes a slight decrease in size (6 mm. x $4\frac{1}{2}$ mm.). This is coincident with a decrease in the parenchymal cells and an increase in the connective tissue and neuroglia elements (figs. 9, 10, and 11). The decrease in size is due probably to contraction. The neuroglia fibers appear continuous with the connective tissue at the periphery of the follicles. They are at least very intimately associated or interwoven, in these regions. Corrosive acetic preparations stained with iron-haematoxylin, which stains the neuroglia fibers dark brown or black, combined with Mallory's connective tissue stain, which colors the connective tissue elements blue, shows the point of transition from one to the other. In many preparations these appear to be in absolute continuity. This staining combination reveals clearly the difference in chemical nature between the true connective tissue and the finer neuroglia framework of the parenchyma. The transition area frequently gives an intermediate color reaction. Only a few of the peripheral cells contain the melanic granules. A few of the central cysts contain acervulus, and there are several spherical areas of dense neuroglia network almost void of cellular elements.

PINEAL BODIES OF OLD SHEEP

Pineal bodies of sheep of the third year are characterized by several degenerative changes; viz.: (1) great increase in the connective tissue elements; (2) large and numerous areas of dense neuroglia network free of cells; (3) areas of apparently coagulated fluid matter; (4) large clumps of intercellular pigment granules in the peripheral portion; (5) comparative rarity of spherical inter-neuroglia cells. The histologic characteristics point to a cessation of active function and to the onset of degeneration. Judging from the standpoint of histologic and cytologic features, the assumed specific function of the pineal gland is important, perhaps essential, only during the first year of life.

GENERAL

Regarding the question as to whether the connective tissue framework of the pineal body is in the form of complete or incomplete anastomosing septa (trabeculae), the evidence in sheep favors the conclusion arrived at by Dimitrova in the ox by the digestion method, viz.: that it consists of trabeculae. This is more particularly true of the main pial extensions as is clearly demonstrated in transverse sections which are circular or oblong in outline (fig. 6). On the other hand, the terminal extensions seem more of the character of reticular sheets of tissue or trabecular network. Studnicka's ('05) suggestion, that possibly in the earlier stages of development, the connective tissue framework of mammalian pineal bodies may actually have the form of septa, as in adult birds, does not express the actual condition in the sheep. Here the earliest pial extensions, as noted for the 10 cm. stage of development, have the character of broad trabeculae.

The blood-vessels follow the ramifications of the pial extensions. There is no question of precedence. The septa are already vascular at the period of first invasion; and the blood-vessels grow as the trabeculae lengthen. The blood-supply comes from the pial vessels which are in union with the blood-vessels of the tela choroidea anteriorly. An ependymal membrane continuous with the latter covers the antero-dorsal aspect of the pineal body. The veins follow the same course through the trabecula as the arteries. Thus cross sections of the coarsest trabeculae show a pair of vascular comites. The artery has much the thicker wall, containing a small amount of smooth muscle, and the smaller calibre. The tips of some of the larger trabeculae contain capillary plexuses which end in open spaces in the centre of follicular collections of cells. The finer trabeculae and septa contain progressively more delicate blood-vessels. In many cases a loop of capillaries forms the centre of a follicle. Another conspicuous feature in connection with the vascular trabeculae is the wide space which exists between them and the surrounding parenchyma. This is spanned by a delicate network of connective tissue fibers which color blue in Mallory's stain, in contrast to the brown or black outlying neuroglia fibers. The meshes are filled with a coagulum. These spaces appear similarly pronounced in pineal bodies treated with any of the above mentioned fixing fluids; also in those of the foetal stages after half term. They can accordingly not be interpreted as fixation artefacts, but are more probably lymph-spaces.

The common cytologic characteristics of the several morphologic types of parenchymal cells (oval, flattened, stellate, and fusiform) are as follows: absence of cell membrane; vesicular nucleus with a plasmosome, and many scattered chromatic granules; occasional presence of melanic granules; otherwise homogeneous or very finely reticular character of the cytoplasm. Atypically, some of the 'oval' cells may have short processes, these resembling stellate cells lying in the meshes of the network formed by the latter; or very rarely a cell (more usually a fusiform type) may have a chromatic nucleus, a condition more probably indicative of preparation for mitosis. In general the nuclei of neuroglia cells are darker than those of the inter-neuroglia cells—and the more highly differentiate types of the former (i.e., with coarse neuroglia fibers and flattened bodies) are frequently very chromatic (fig. 8).

REVIEW OF LITERATURE

Only Flesch ('88) and Dimitrova ('01) in their comparative studies of the pineal body of various mammals have paid special attention to that of sheep. But Flesch studied more particularly the epiphysis of the dog and the bat, and Dimitrova reports more fully on appearances in ox, calf, cat, and man, observing that the body of the sheep is very similar to that of the ox. This general neglect to make a careful and detailed study of the pineal body of the sheep is the more remarkable when one recalls its large size and its easy availability.

Flesch argues for the non-rudimentary condition and for an important physiologic significance of the adult pineal body in man and mammals on the basis: (1) its nerve supply (from optic thalamus; Kölliker, '96); (2) peculiar general characteristics (lobulation; pigmentation); and (3) presence of specific cells. To these might well have been added a fourth point; viz., its great vascularity. Flesch notes lymph cells in process of transit from the pineal recess through the lining ependyma, and their presence in the coagulated content of the recess; neither of which observations I can confirm. Flesch summarizes the main evidence in support of the secretory significance as (1) the large amount of coagulum in the recessus pinealis, and (2) the presence of pigment granules in the specific cells; and in opposition thereto as (1) absence of a specific reaction of the parenchyma which would indicate a specific chemical activity, (2) no correspondence between size of gland and central nervous system nor to size of body as a whole. He notes that in spite of small size of sheep's brain the pineal body is relatively large. It is about half as large as in man, according to Flesch, but of about the same size according to my observations. In view of the above considerations, more particularly on the basis of the presence of pigment, Flesch is inclined to regard the pineal body of mammals as a modified sense-organ which has lost its primitive visual function. Regarding the question of a possible secondary function, we stand here before a riddle which can only be solved by experimental methods of investigation.

Dimitrova ('01) gives an excellent review of the earlier literature on the mammalian pineal organ. She credits Cionini ('88) with having first demonstrated the neuroglia nature of the pineal body. Galeotti ('96) appears to have been among the first to argue cogently for a secretory function of the pineal organ. According to Galeotti the cells of the parenchyma elaborate pigment and, by a different mechanism, a product of secretion in the formation of which nucleus and nucleolus participate. He also seems first to have suggested that the pineal body is predominantly neuroglia in nature, the several parenchymal elements being variously modified ependymal cells. This is also Dimitrova's conclusion, in opposition to that of previous investigators who recognized in the parenchyma two or three specific types of cells.

Dimitrova describes cavities lined by cylindrical cells in ox, calf, sheep, and dog. Both the cytoplasm and the nuclei of the parenchymal cells are said to contain granules. Dimitrova does not attempt to decide as to whether they have a secretory significance or whether they build neuroglia fibers, or whether they represent degeneration products. She describes nerve fibers in relation to the blood-vessels; but was unable in the adult to recognize them with certainty in the parenchyma "among innumerable fibers which impregnate with silver chromate." Nor can she confirm Cajal's observation concerning the presence of sympathetic cells.

In Oppel's Lehrbuch, Studnička ('05) gives a masterly brief summary of our knowledge regarding the structure of the mammalian pineal body. The appended bibliography includes a complete list of the literature on the vertebrate pineal organ.

NATURE OF THE CYTOPLASMIC GRANULES

The sole granular elements of the cytoplasm that I have been able to demonstrate with the aid of all the different technical methods above mentioned, are equally well discernible in unstained preparations of formalin-fixed material. They have a uniform spherical shape but vary considerably in size, the larger being approximately ten times the bulk of the smallest (fig. 4 *b*).

They vary in color from a light yellowish or greenish brown to a deeper brown or even black. Occasional rod and ring forms appear. The larger granules are the darkest. The smaller have a metallic luster. When abundant, as in the corpora pinealia of half-term sheep, they give a grayish or black color to that body. Their color is deepened by osmic acid, and the various haematoxylin stains, indicating a partial lipoid nature. They are distinctly cytoplasmic (i.e., extra-nuclear). In no case can similar granules be seen within the nucleus. The latter, in unstained preparations, appears homogeneous; in stained preparations the numerous chromatic granules of the nuclear reticulum simulate these black cytoplasmic granules. I can find absolutely no cytologic evidence for other secretory products to which, according to Galeotti, the nucleus and nucleolus contribute. Nor is there any evidence for a transit of melanic granules from nucleus to cytoplasm. Since stained preparations reveal no cytological features (granules) not conspicuous in unstained sections, I cannot accept the view that such are present in the sheep

The nucleus contains only chromatic granules; the cytoplasm only melanic granules. Perhaps the granules illustrated and described by Dimitrova for Bos taurus, and the granules designated by Galeotti ('96), are of the same nature as those here described for the sheep. Morphologically they appear identical. Dimitrova's illustration of a pineal cyst in Bos taurus, lined with irregular cylindrical cells with a few spherical granules distally, can be paralleled by innumerable examples from my specimens from the sheep (fig. 6 and 7), in which latter case the granules are undoubtedly melanic. None of the three interpretations suggested by Dimitrova (i.e., secretion, degeneration or mitochondrial granules) seems to accord with the facts. They cannot be seen building the neuroglia fibers, hence most probably they are not mitochondrial. Moreover they are absent in the cells of the pineal body of the opossum where very coarse neuroglia fibers appear. Nor have they been described in the histogenetic process of neuroglia formation in the neural tube, where Hardesty ('02 and '04) regards neuroglia, in elephant and pig, as differentiated spongioplasmic fibers. Since they are more abundant in the young and actively growing bodies, and present in dividing cells, they cannot be regarded as products of degeneration. Since they appear to be true melanic granules they cannot be interpreted as secretory in the stricter sense of the word. They make their appearance as such first in the cytoplasm, under nuclear influence probably, but apparently not as nuclear extrusions. In the pineal body of the embryo sheep they appear only in small quantities in only very few cells; at half term they are more or less abundant in almost all the cells. They are interpreted as melanic mainly on morphological and optical grounds, and on the basis of negative microchemical tests; i.e., they do not turn blue in a 2 per cent acidulated potassium ferricyanide solution, hence not haemosiderin, nor iron-containing; nor red in Sudan III, hence not predominantly lipoid in nature.

These granules must accordingly be regarded as most probably melanic. What then is their significance? I must again emphasize the fact that their decrease in later stages is probably only apparent and not real. There are relatively fewer granules per cell in consequence of a mechanical distribution, by mitosis, among the more or less numerous descendants of the original pigmented cells. The quantity of pigment in the final descendants thus depends upon the endowment of the original ancestor, and the number of intervening generations. Possibly small additions are made in occasional generations and the large mass of pigment granules in old glands seem due to local unusual (primitive?) conditions stimulating to excessive pigment production. Regarding the significance of this voluminous pigment production among the parenchymal cells of the foetal pineal body, two more reasonable interpretations suggest themselves. The one must be expressed in terms of the biogenetic law; the other in terms of nutrition. The second assumes that melanin is chemically proteid plus lipoid in varying proportions and capable of transformation into usable food material. But it appears the less valid since the granules do not increase concomitantly with growth, nor ever wholly disappear. Assuming that the mammalian pineal body represents the epiphysis of reptiles, or other progenitors in the direct mammalian ancestry, where it functioned as a visual or light perceptive organ, the production of pigment in the homologous cells of this mammalian pineal vestige becomes intelligible in terms of an hereditary attempt to subserve phylogenetically earlier functions. The nature of the stimulus that incites to such great post-natal proliferative activity in some forms is the riddle that baffles speculation.[1] As above stated, there is discernible no further evidence for a secretory function. The vacuoles noted by Dimitrova—present also in the cells of the sheep's pineal body—do not seem to me to have the value of vesicles of a specific secretion (colloid?) as suggested by Dimitrova. The smaller and more nearly spherical cells have a homogeneous cytoplasm. The larger and more irregular cells are the more vacuolated and reticular. The vacuolization appears to be due to a mechanical adjustment of the cytoplasm to enlarging confines; a sign of age (or differentiation) or degeneration.

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[1] A subsequent search for possible secretory granules in pineal bodies of young sheep, by means of the Altmann technic, has revealed a granular character of the cytoplasm in material so treated. Samples from the same pineal body fixed in a 10 per cent formaldehyde solution show the usual very finely granular or homogeneous structure of the cytoplasm of the parenchymal cells. The more coarsely granular character of the cytoplasm thus seems due to the action of the modified Flemming's fluid (equal parts of a 2 per cent solution of osmic acid and a 5 per cent aqueous solution of potassium dichromate), and is more probably of the nature of an artifact. The Altmann technic, however, reveals the presence of certain lipoid bodies which were lost by all the other methods of preservation employed. In samples of the same body (perfectly fresh), fixed in formaldehyde, these were missing. They were lost also in material which was differentiated in the picro-alcohol solution for more than a fraction of a minute or passed too slowly through the alcohol in dehydrating for mounting. These bodies, accordingly, are highly soluble lipoids, which disappear in all ordinary technics where alcohol is used. The occasional presence of a few light brown or yellowish melanic granules, contrasting sharply with the dark brown and black color of the lipoid granules, leaves no ground for possible confusion. The granules in question are larger and smaller spheres, the majority of which seem to be disintegrating giving rise to spherical masses of very small black granules. The original 'sphere' is represented by a lighter substance in which the smaller granules are embedded. Some spheres contain only a few granules. The solid lipoid sphere seems to pass through this as an intermediate stage to one of more fluid form.

The vacuoles seen in the cytoplasm of the parenchymal cells of the same tissue treated with the formalin technic undoubtedly represent the lipoid bodies after solution, and this is probably the origin also of at least some of the vacuoles occasionally seen after treatment with the usual technics involving the relatively prolonged use of alcohol. These occasional lipoid bodies probably indicate intracellular degenerative changes, and are not mitochondrial nor secretory in nature.

CONCLUSIONS AND DISCUSSION

The pineal body of the sheep undergoes its greatest development (five-fold) during the first year of life. After this period regressive changes make their appearance. Its general structure (i.e., lobulation, connective tissue framework, arrangement of parenchyma into follicles, presence of blind alveoli, large peri-vascular lymph spaces, great vascularity, and presence of granules in the cytoplasm), indicates a glandular function of the nature of elaborating an internal secretion. The coincidence of greatest developmental activity with greatest body growth and the appearance of sexual maturity indicates a casual nexus. However, there is no cytologic evidence in support of this opinion. The parenchymal cells are all of one type: more or less highly differentiated ependymal cells, giving origin to neuroglia cells and fibers, and interneuroglia cells (figs. 8, 10, and 11). The cytoplasmic granules are evidently melanic and represent the morphological expression of a hereditary impulse to function as their ancestral homologues in the median visual organ, the parietal eye.

A priori, one inclines to believe that so well developed and large a structure, compared with the brain of sheep, must have a specific and important physiologic function. But the histologic evidence in confirmation of this impression is almost *nil*. The effects of intra-venous injections of pineal extract are definite, but hardly pronounced or specific enough to have vital significance. Of course, the assumed specific internal secretion might indirectly affect the body metabolism and so be highly important for health and normal growth. This matter can only be fully elucidated by extirpation experiments. The only significant fact from the standpoint of function revealed by the developmental history of the pineal body in the sheep is that, whatever possible function it may have in the animal economy, this is most active during the first eight months of post-natal life.

It remains to discuss the fate of the numerous cysts of the second half of the foetal stage of development. Their abundant presence makes particularly striking the similarity between the pineal body of this stage and an alveolar gland. Their disappearance in large part during early post-natal stages (very few remain at 8 months) indicates that the assumed glandular activity prevails only during foetal life. These 'acini' are filled with coagulated fluid, which may possibly be of the nature of an internal secretion. However, their origin as above described, shows that they are actually of the nature of cysts, and make it very improbable that their fluid content has secretory value. Contrary to Flesch's opinion (cited from Studnička) they are not lined with ependymal cells from the recessus supra-pinealis, nor have the lining cells a distinct basement membrane. The disappearance of these numerous 'acini' is accompanied by the more active proliferation of post-natal life obliterating the space by simple pressure on the surrounding cells. Proliferation of cells in the actual wall of these acini is a minor contributory factor in their obliteration. Since these acini arise as cysts (i.e., independently of recess or surface) in a healthy and actively growing structure, and disappears again in large measure when the pineal body attains its greatest bulk, their significance in terms of function is very obscure. They become more intelligible when viewed from the phylogenetic standpoint. They may be interpreted as ontogenetic representative of true alveoli in the glands of phylogenetically earlier ancestors.

Finally, it seems important again to emphasize the fact of a close structural similarity (practical identity) between the pineal bodies of late foetal ($4\frac{3}{4}$ months) and early post-foetal life (to 8 months); also the uniformity in size, form and texture of the pineal bodies of young sheep (first year), and the considerable variation, both local and general, among the pineal bodies of older sheep. The variations designated indicate degeneration and the abatement of any possible active function.

SUMMARY

1. The pineal body of the sheep begins its development as in other forms as an evagination from the roof of the diencephalon. At the 2.5 cm. stage of development it has the form of a definite pocket lined with ependyma thickened apically.

2. At the 5 cm. stage the cells of the distal and dorsal walls have become stellate with anastomosing processes forming a loose-meshed syncytial network. This represents the first stage in the formation of the neuroglia framework. The nuclei of the cells are vesicular, with plasmosome and scattered chromatin granules. The cytoplasm is homogeneous or reticular (with occasional vacuoles). A few of the cells contain a few melanoid (melanic) granules.

3. The body takes a backward direction in growth. The apical cells proliferate more actively, thus forming a rounded dome-shaped body with shallow proximal pineal recess continuous with the third ventricle. Blind alveoli (cysts) make their appearance. The pia mater sends in vascular trabeculae dividing the body into more or less distinct lobules and follicular compartments of parenchymal cells.

4. These same processes continue to half term (21 cm.) when proliferation seems most active. The pigment granules are now most abundant, and the 'alveoli' are largest and most numerous.

5. Pineal bodies at birth are similar to the above, only larger. There are besides, follicular collections of cells with central vascular trabecula or capillary network, surrounded by a wide perivascular lymph-space.

6. Between birth and the end of the first year the pineal body enlarges approximately five-fold. The pigment granules have been distributed by mitosis of the containing cells among many descendants thus giving the impression of a numerical decrease. Vascular follicles are abundant. An occasional cyst remains. Medullated nerve fibers can be traced into the basal portion and in relation to the blood-vessels. The parenchyma consists of a single cell type; a more (neuroglia cell and fibers) or less (interneuroglia—spherical or polygonal) differentiated ependymal cell. The cysts have disappeared in consequence of crowding of the proliferating enveloping cells.

7. After the first year local signs of degeneration appear, i.e., increase of connective tissue and neuroglia, and the appearance of brain-sand, large clumps of pigment granules, cell-free areas of oedentatious neuroglia network, and a decrease of parenchymal cells.

8. There is no clear histologic evidence indicative of a glandular function.

9. If the pineal body in the sheep subserves an important physiologic function, this is probably active only during the first eight months of post-natal life.

10. The sole cytoplasmic granules are melanic and probably have only ancestral significance. The cysts are similarly interpreted.

11. The neuroglia fibers appear to have their origin in the thickened and fused spongioplasmic fibers.

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PLATE 1

EXPLANATION OF FIGURES

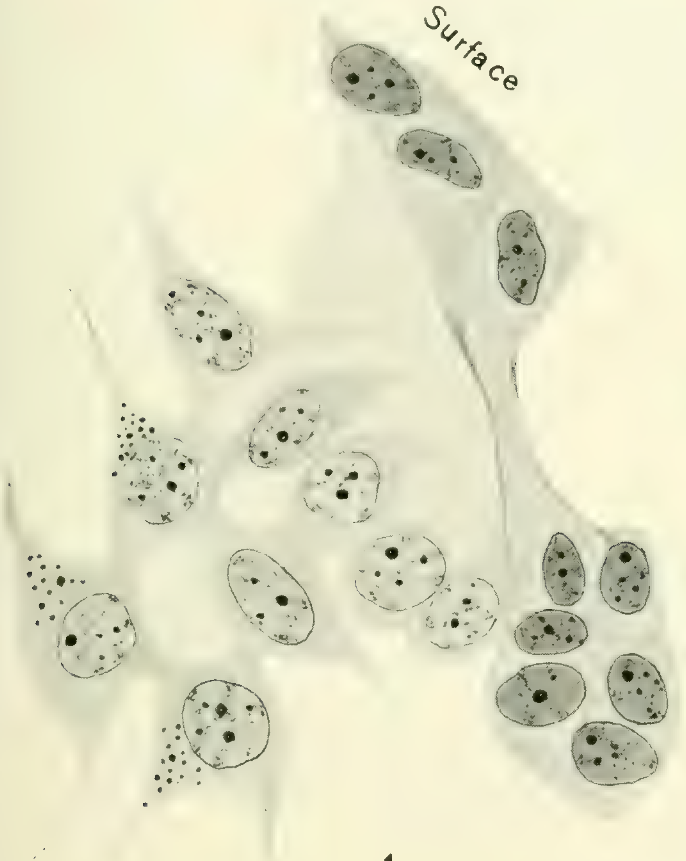
1 Dorsal peripheral region of pineal body of 5 cm. stage of development showing the early stages in the process of neuroglia formation. × 1500.

2 Middle region of pineal body of 10 cm. stage showing neuroglia cells, the thick walled character of the smaller blood vessels, and the large perivascular (lymph?) spaces. × 1500.

3 Central cells from 10 cm. stage of development, showing the transition from ependyma to neuroglia cells, and the distribution of the melanic granules in each. × 1500.

4*a* Two neuroglia and one inter-neuroglia cells from 11 cm. stage of development.

4*b* Various forms and sizes of the melanic granules. × 1500.



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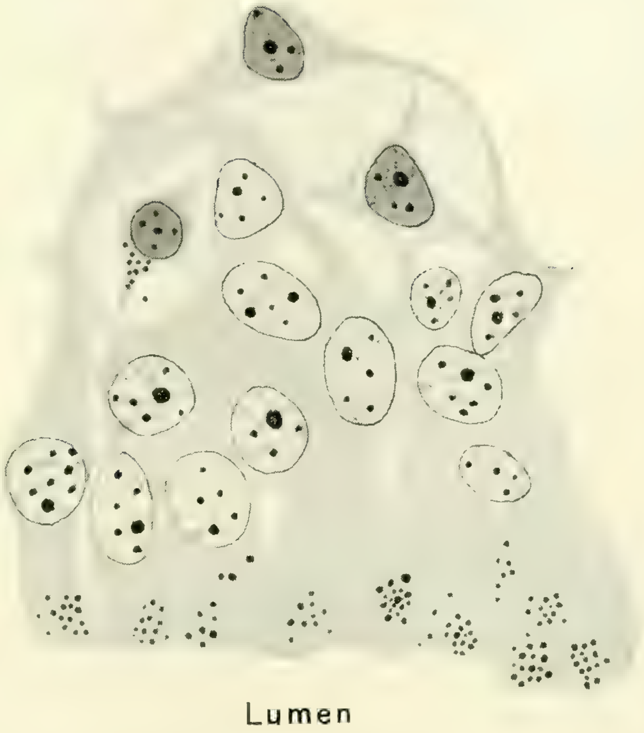

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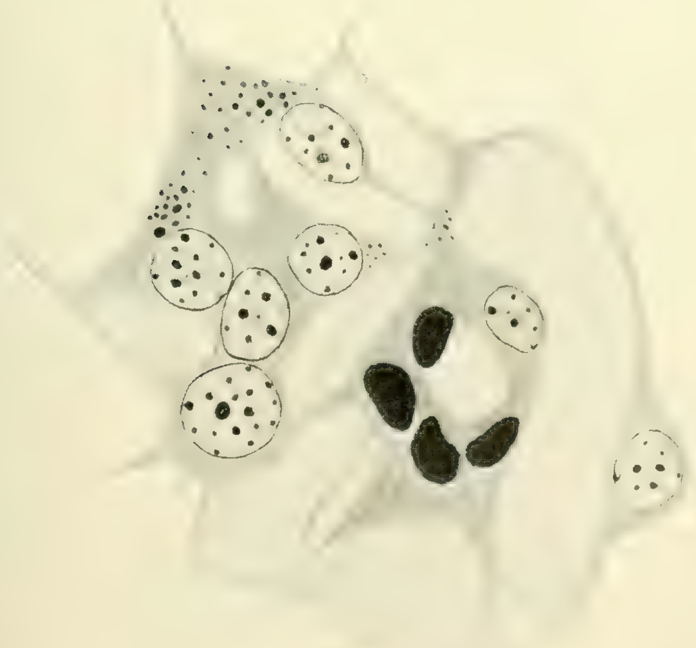

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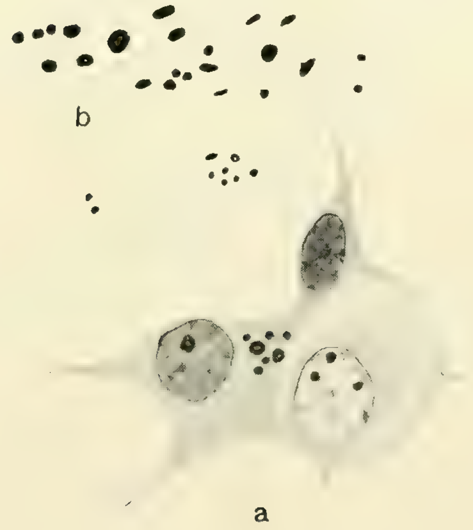

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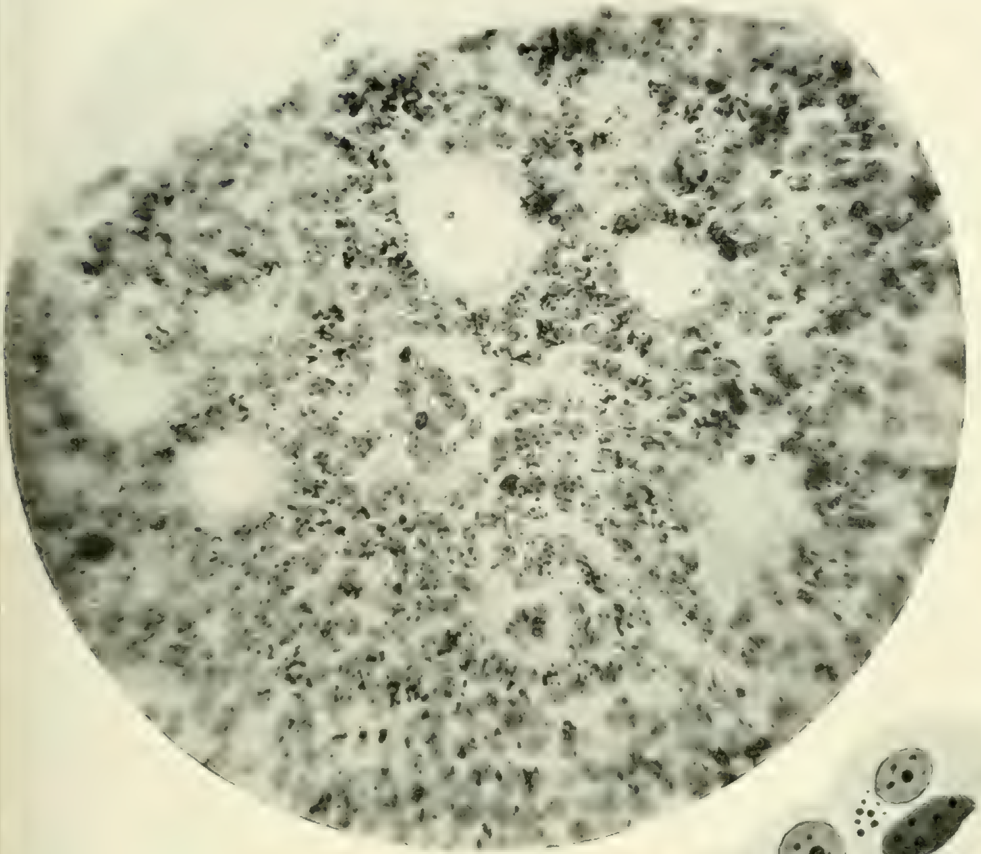
PLATE 2

EXPLANATION OF FIGURES

5 Cyst invaginated by a vascular pial trabecula, forming a ‘glomerulus’ from 15 cm. stage of development. × 1500.

6 Photomicrograph of peripheral portion of pineal body of 21 cm. stage, showing several cysts and several vascular trabeculae, and the presence of an enormous number of melanic granules. × 250.

7 Transverse section of a small alveolus or cyst, showing the character of the cells, the distribution of the melanic granules and the reticular (coagulated) content. × 1500.



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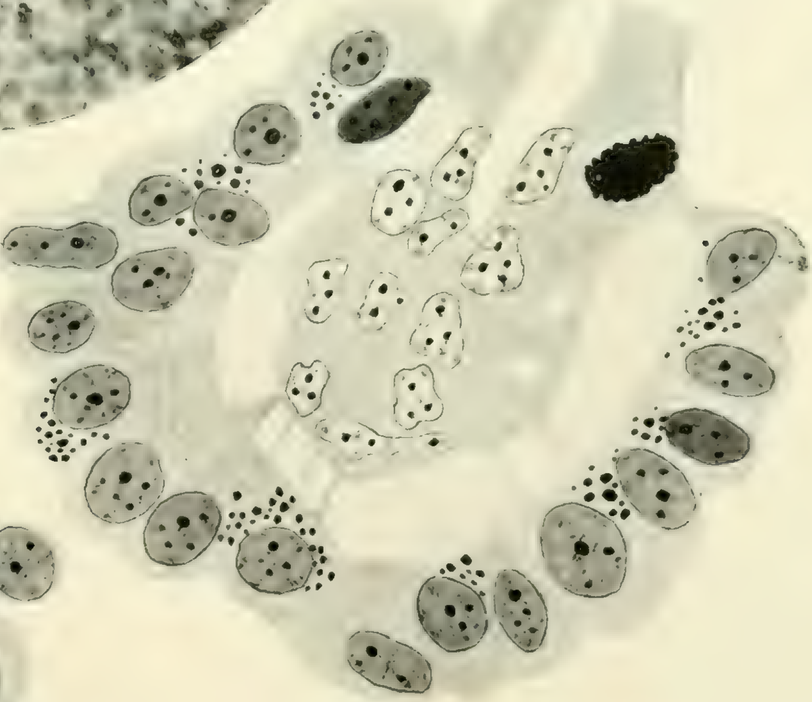

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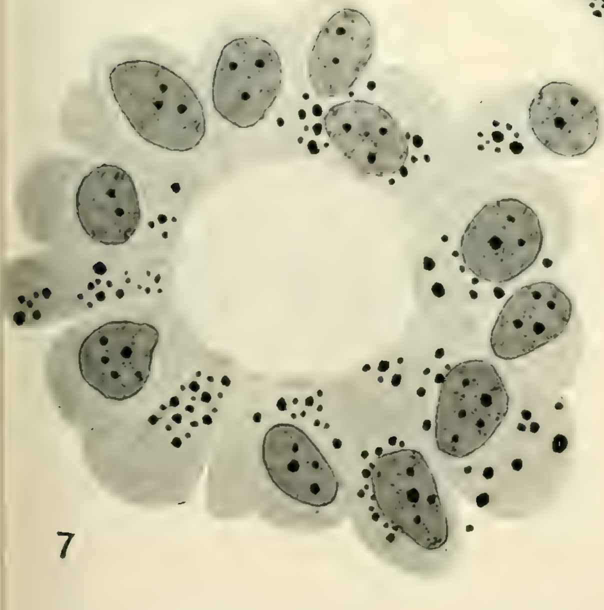

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PLATE 3

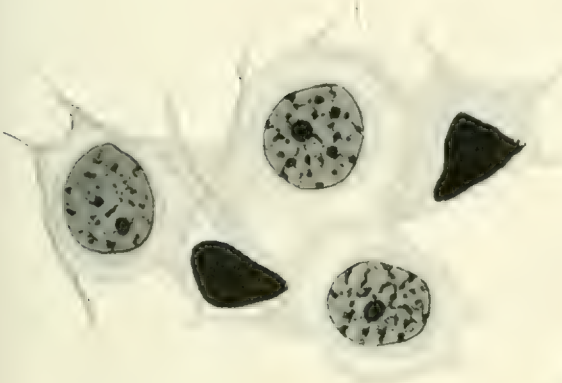
EXPLANATION OF FIGURES

8 Two neuroglia and three inter-neuroglia cells from pineal body of lamb. × 1500.

9 Photomicrograph of peripheral region of pineal body of yearling sheep to show the character of the parenchyma, neuroglia cells and fibers, and inter-neuroglia cells.

10 Neuroglia cell with fibers, and inter-neuroglia cell with melanic granules from yearling sheep. × 1500.

11 Nueroglia cell with adjacent fibers from pineal body of a yearling sheep. × 1500.



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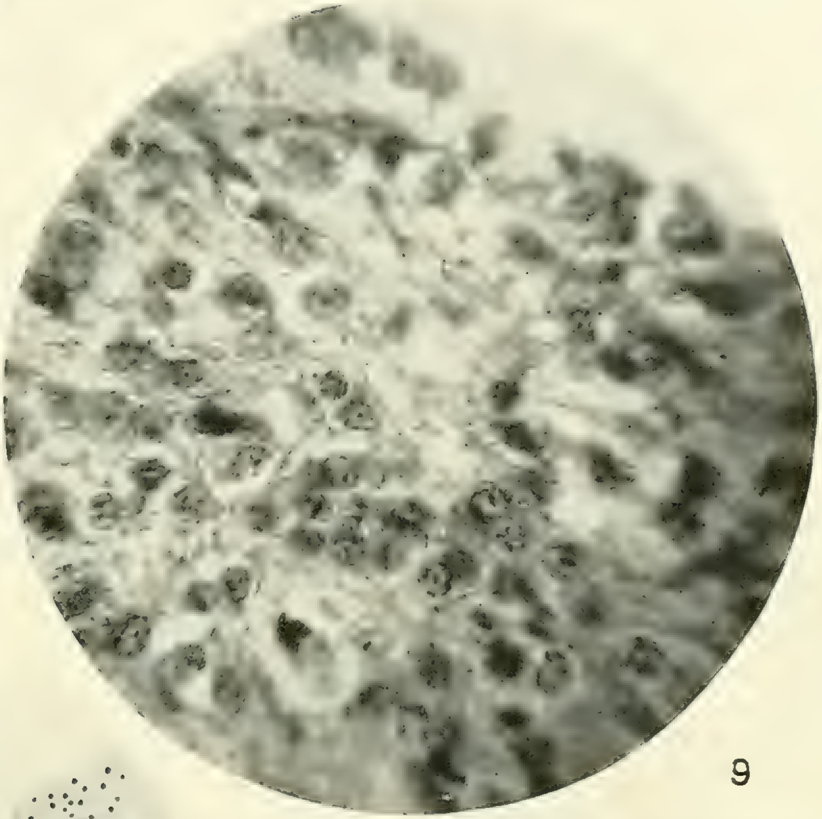

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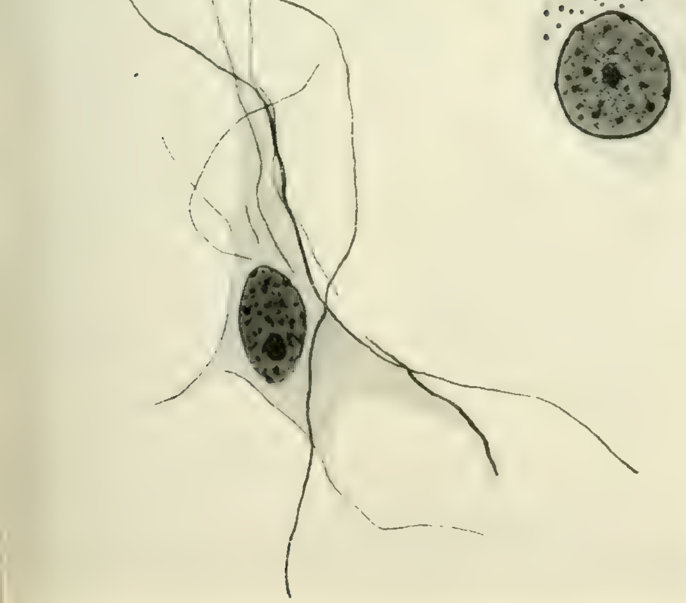

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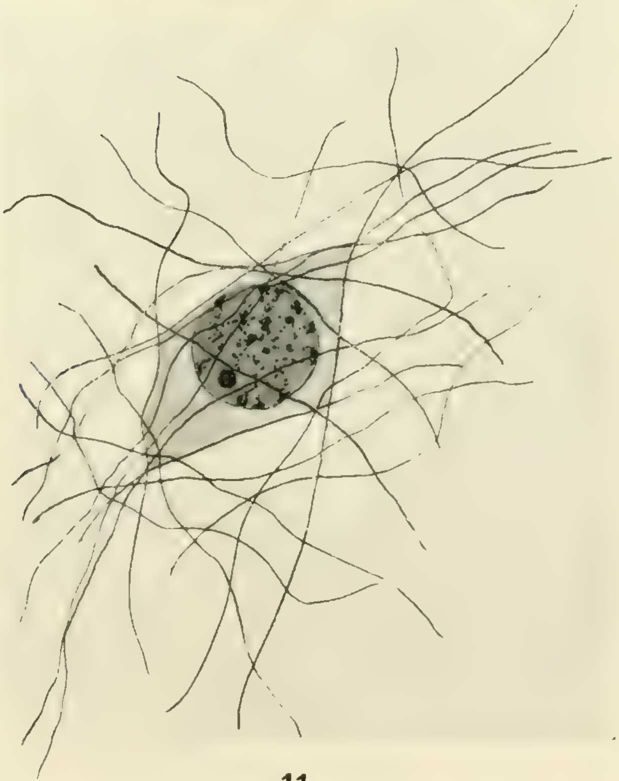

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THE APPLICATION OF THE SILVER IMPREGNATION METHOD OF BIELSCHOWSKY TO RETICULAR AND OTHER CONNECTIVE TISSUES

1. THE MATURE TISSUES

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THIRTEEN FIGURES

The minute structure of true reticular tissue (reticulum, Mall) has long been a matter of more or less controversy. Kölliker and his followers regarded it as composed of branching, anastomosing cells, while Bizzerzo, recognizing and laying more stress upon its fibrous character considered it composed of bundles of fine fibers to which the fixed connective tissue cells are closely applied. As Mall ('96) has pointed out it makes but little difference whether the fibers are within or without the cells provided we understand what is the precise relation.

A few fundamental facts of structure may be taken for granted. That reticulum contains both fibers and fixed connective tissue cells is obvious. That it is more or less infiltrated by leucocytes is well known. That its anastomosing elements are frequently continuous with bundles of white fibers may be readily observed in any section of lymphoid tissue. The points of divergence appear when one attempts to determine the relation: (1) of fixed connective tissue cells to reticular fibers ('Gitterfasern'), (2) of reticular fibers to elastic fibers, (3) of reticular fibers to the white fibers of collaginous tissue, (4) of 'fixed' to 'wandering' cells. The present paper is concerned with the attempt to throw some light upon the basic problems related to the first three of these questions.

The early studies of Kölliker, Ranvier and others, were largely conducted upon teased and unstained reticulum or upon lymphoid tissue from which the lymphocytes had been washed out by various methods. They were followed by the employment of the more recent dye reactions by which the recognition of the fibrillar character of the tissue is rendered somewhat more apparent. It was not until the more exact methods of chemistry and microchemistry were applied by Siegfried, Mall and others that a fairly clear perception of the exact structural relation of the reticular tissue began to be apparent. The introduction of new methods often renders plain certain hitherto obscure facts. This is especially true as a result of the silver impregnation methods of Bielschowsky ('04) when applied to various connective tissues. Even the finer fibers, which are more or less obscure after preparation with other methods, stand out clearly in these preparations and one is thus enabled to draw sharper distinctions than is otherwise possible. It is with the application of this method, and its modification by Maresh ('05), that my observations were largely made; the results have been confirmed by comparison with consecutive serial sections stained by well known methods, chiefly depending on haematoxylin and eosin, Mallory's fibroglia stain, and the combination of haematoxylin with the Weigert and Van Giesen stains described in my Textbook of Histology ('05). The tissues studied have been lymphatic glands, spleen, tonsil, thymus, the lymphoid tissues of the digestive and respiratory tracts, the skin and various other tissues being used for comparison. The material was obtained from man, pig, dog, cat, rabbit, ox, sheep, calf and fish. It was fixed by the various methods in common use and was both mature and embryonal.

The method of impregnation which I have followed has been a variation of the rapid modification described by Maresh ('05). With individual exceptions I have gotten uniformly good results after all the methods of fixation used. The method was applied as follows:

1. Sections cut in paraffin were fixed on the slide and placed 12 to 24 hours in a 2 per cent solution of silver nitrate.

2. Transfer for 15 to 30 minutes to freshly prepared alkaline silver solution (20 cc. of 2 per cent silver nitrate to which are added

three drops of 40 per cent caustic soda and the precipitate redissolved by adding ammonia drop by drop while stirring).

3. Rinse quickly in distilled water and place in 20 per cent formalin for three minutes or till the sections are black.

4. Wash in distilled water and place for ten minutes in an acid gold-bath (10 cc. distilled water to which are added 2 to 3 drops of 1 per cent gold chlorid and 2 to 3 drops of glacial acetic acid).

5. Immerse in 5 per cent hyposulfite of soda $\frac{1}{2}$-1 minute to remove all unreduced silver.

6. Wash in distilled water, dehydrate, clear in xylol and mount in balsam.

Bielschowsky advised leaving tissues in the 20 per cent formalin for 12 to 24 hours but as Maresh has shown this seems to be unnecessarily long, at least when sections are used. To still further shorten the process Woglom ('09, '10) has advised, for the purpose of preventing shrinkage of the tissue, that the initial immersion in 2 per cent silver nitrate solution need not exceed 5 minutes. I have, however, found this time entirely too short in many cases and its use led to much confusion in the interpretation of my early results. In sections insufficiently impregnated the contrast between collaginous and reticular fibers was not sharp, either the reticulum taking a brown instead of a proper black color in lightly toned preparations or if the toning was intensified many of the collaginous fibers became a greyish black instead of the proper golden brown. Moreover, I did not find troublesome shrinkage of tissue in well fixed preparations. I would therefore advise a strict adherence to the 12–24 hour period of immersion recommended by Bielschowsky and Pollock ('04), Levi ('06) and others, rather than the shorter period advocated by Woglom and, with reservations, by Maresh ('05).

In the lymphoid tissues the impregnation brings out most distinctly the reticular fibers; they take on a deep opaque black and stand out prominently against the golden brown of the collaginous fibers. The method has already been applied to connective tissues and the assumption of a more or less specific staining property for reticulum in the liver, lymphatic glands, tonsil, spleen, ovary, and pleura has been casually recorded by Maresh ('05), Ciaccio ('07), Alagna ('08), Balabio ('08), Cesa-Bianchi

('08), and Favaro ('09). Realizing the uncertainty which attends the use of various silver methods one readily appreciates the necessity for careful study of the effects of the method upon the various tissue elements. Of the primary tissues epithelium and other cells are scarcely if at all colored or have a faint brownish tint; red blood cells darken readily and are either opaque black or a deep brown according to the depth of the impregnation and the duration of the toning bath; blood serum and intercellular cement substance blacken, the latter appearing very granular; all nuclei are an intense black, the silver reacting specially to the chromatic portions, viz., nuclear wall, chromatin net and karyosomes; the axis cylinders of nerves are somewhat blackened, though with the method employed the neuraxes are not nearly so opaque as the fibers of reticular tissue. Muscle fibers blacken irregularly depending on the depth of impregnation and they show something of their fibrillar structure; the cross striations and, in smooth muscle, the myofibrillae and intercellular bridges appear beautifully shown in certain instances but it is possible with care to have the muscle almost colorless and the reticular fibers an opaque black. The silver apparently adsorbs somewhat to the surface of muscle cells and elastic fibers and thus frequently fills the interstices between fibers, forming an apparent interfibrillar network in smooth muscle, in epithelium and in dense elastic tissue, e.g., ligamentum nuchae. It is possibly this which accounts for the apparent blackening of intercellular substance. Both because of their reactions and because of their characteristic differences of structure one has little difficulty in differentiating these tissues after impregnation and distinguishing them from the various types of connective tissue fibers.

Before we can regard the method of Bielschowsky, applied to tissues outside of the nervous system, as a specific stain for reticulum, it is necessary to examine more carefully than has been done, into the reaction of connective tissue fibers to the silver impregnation and the differentiation, in sections prepared by this method, of the collaginous, elastic, fibroglia and reticular fibers. Of the fibrous tissues cartilage, bone, and dentine may be set aside because of their characteristic structure, obvious at a glance, though one sometimes encounters difficulties in the transition from the fibrous perichondrium to the cartilage matrix.

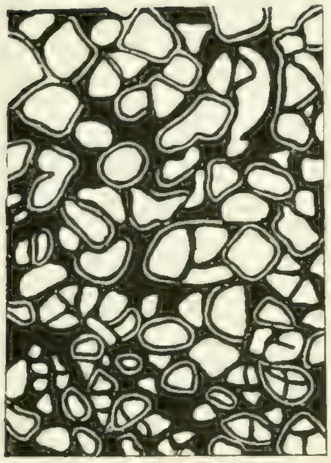
The distribution of the fibers blackened by silver is so extensive that one is tempted to question the selective action of the method. They are encountered in all the lymphoid organs, in the mucosa of the digestive tract, in and about the walls of the lymphatics and blood-vessels, in the framework of all the secreting glands, e.g., liver, salivary glands, pancreas, mucous glands of the respiratory and digestive tracts, in the kidneys, ovary, uterine wall, testis, prostate, sweat glands, in the corium of the skin, in the tunicae propriae of the respiratory and digestive apparatus, about the glands of the gastro-intestinal mucosa, and to a limited extent among the fibers of areolar and collaginous tissue wherever it is found. Many of the basement membranes consist largely of these argentiferous fibers.

If one is careful not to overtone the specimens the results in mature tissues are fairly constant; in such preparations (with a few reservations) the method appears quite definitely selective for the blackened fibers of reticular tissue ("reticulum." Mall), the elastic and fibroglia fibers remain colorless and the collaginous fibers assume a brownish tint.

This result was arrived at by a careful comparison of the effect of this and other stains upon the several tissues in locations where each is known to occur. The conclusions are based on the following observations.

A. ELASTIC FIBERS

Sections of the ligamentum nuchae after impregnation show the elastic fibers absolutely colorless and outlined by an intense black fibrous mass which occupies nearly the entire non-vascular area between the elastic fibers, and which at the borders of the elastic bundles shades into the golden brown of the collaginous fibers forming the coarse bands of the framework (fig. 1). In the controls the elastic fibers show the characteristic staining reaction with hematein and eosin and with Van Giesen's stain, and the intervening tissue is colored red by eosin and by acid fuchsin.



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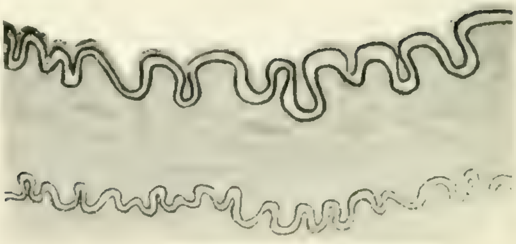

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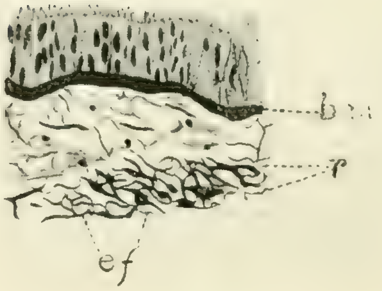

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Fig. 1 Transection of the ligamentum nuchae of an ox. Bielschowsky stain. The elastic fibers are colorless, the intervening tissue opaque. Camera lucida; occ. 1, obj. $\frac{1}{6}$.

Fig. 2 A pulmonary artery from the human lung showing the colorless internal and external elastic membranes invested by blackened reticulum. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{6}$.

Fig. 3 From a primary bronchus of man. Basement membrane (*bm*) is a dense opaque black, its fibers so closely packed as to be indistinguishable. The elastic fibers (*ef*) are colorless and are invested by a black reticulum (*r*). Bielschowsky stain. Drawn with the Edinger projection apparatus, × 255.

In the fenestrated coats of the arteries one sees in the larger arteries of the lungs, stomach, lymphatic glands and many other organs vessels showing in the controls the typical elastic membrane in the tunica intima completely encircling the vessels in transections. The impregnated specimens, when the same vessel is examined in adjacent slides in the series, show the internal elastic membrane colorless, the elastic coat in the larger vessels being invested on either surface with a close net of black reticular fibers (fig. 2). This investment of the elastic tissue by reticular fibers is readily observed and is most remarkable. Similar, though less numerous fibers are seen investing the elastic tissue in the intermuscular spaces of the tunica media, and in the tunica adventitia.

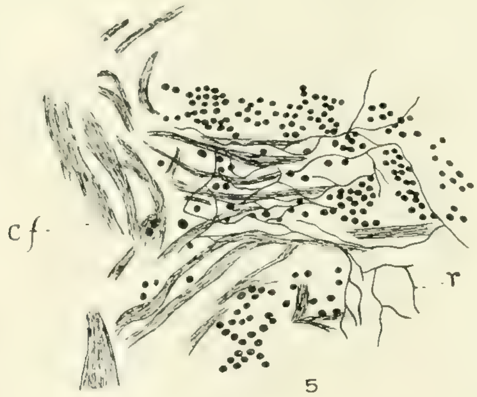
In the smaller arteries and in the small and medium veins the coat of Henle is so thin, and often incomplete, that it is more difficult to determine that the elastic fibers are colorless as distinguished from the blackened reticulum but in view of the constant and obvious condition in the larger vessels one is warranted in assuming that the elastic fibers in the smaller vessels, as in the larger, are colorless and that it is the reticulum, when present, which blackens. The intimate clothing of elastic fibers by reticulum, readily observed in the larger vessels, accounts for the occasional appearance of blackened fibers in the position of Henle's coat in vessels so small as to possess only an incomplete internal elastic membrane.

In the basement membranes of the bronchii one finds only argentiferous reticular fibers. In the larger bronchi the basement membrane is specially distinct and consists of a dense, closely packed mesh of blackened reticular fibers (fig. 3), forming a complete membranous investment continuous with the reticular fibers of the tunica propria and supporting the epithelium. With the Weigert-elastic picro-fuchsin stain the argentiferous fibers take a red color.

In the tunica propria of the trachea and bronchi are large bundles of longitudinal elastic fibers. These fibers remain colorless in the Bielschowsky sections even when the stain has been made so intense as to darken to a considerable extent the collaginous fibers and the muscles. One finds each elastic fiber invested by a distinct coat of blackened reticular fibers forming an intricate net. If one selects a known and readily recognized point for study, consecutive sections stained by different methods show the broad lines of elastic fibers, which in the Bielschowsky sections are colorless, to be flanked on every surface by a blackened reticulum, but clothed in the Weigert-elastic picro-fuchsin section by fuchsin stained fibers. With haematoxylin and eosin the whole breadth of the basement membrane and both elastic and argentiferous fibers in the tunica propria take the characteristic eosin tint, and reticular and elastic fibrils are almost indistinguishable.

Thus wherever the recognition of unquestionable elastic fibers can be made with certainty they are found uncolored by the silver, while giving characteristic reactions to other stains. It is only in those locations where identification of elastic fibers is questionable that one is inclined to suggest their identity with the blackened fibrils, but even then one sees indications of noticeable difference. If a bit of areolar tissue is carefully examined in the wall of the digestive tract, the skin, the peritoneum or elsewhere, and sections of the tissue are also stained by the selective elastic tissue stains, Weigert's, or Unna's, one observes on comparison a difference in the number and arrangement of fibers selected by the compared methods. The orcein and Weigert sections will compare very closely. The Bielschowsky sections of the same series frequently show many more fibers of the blackened type; moreover the blackened or reticular fibers are usually more wavy and of irregular distribution, often having a typical spiral appearance as compared with the relatively straight elastic fibers. This is well shown in sections of collapsed or undistended lung in which the elastic fibers of the alveolar walls and bronchioles are straight while the reticular fibers, from the extreme contraction of the organ, are thrown into a remarkably intricate network of wavy and twisted fibrils, equally as distinct and more abundant than the elastic.

These findings are in confirmation of the views already expressed by Woglom ('10) and others and appear to prove conclusively the non-identity of elastic fibers with those fibers (reticulum) which blacken in these preparations. This view is in accord with that expressed by Mall ('02), who as a result of his comparison of the tissues by chemical methods likewise demonstrated the non-identity of elastic fibers and reticulum, but his studies of mesenchymal tissues showing embryonic stages of the connective tissue, resulted in pictures delineating the first appearance of elastic fibrils which simulate those which I have obtained in similar tissues by the method of impregnation (fig. 4). I shall consider this phase of the subject in a later paper. At this time it is sufficient to say that I consider the fibers referred to to be collaginous in type. One must therefore finally emphasize the fact that in well recognized portions of mature tissues elastic fibers in the silvered preparations remain entirely colorless while reticular fibers



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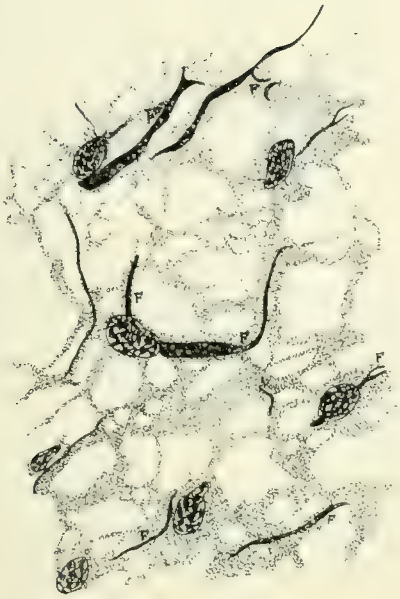

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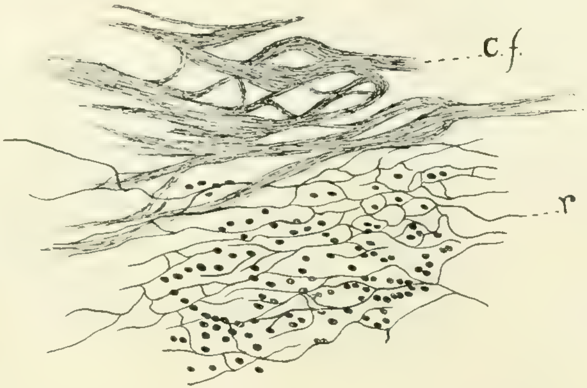

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Fig. 4 From the subectodermal mesenchyme (corium) of a fetal pig of about 80 mm. neck-breech length, showing blackened fibrils (F) bearing intimate relations to the mesenchymal cells. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom. im.

Fig. 5 Reticular and collaginous tissue at the periphery of a lymphatic gland. Observe the sharp outlines of the fine black reticular fibrils (*r*) which intermingle with the bundles of collaginous fibers (*cf*) in a trabeculum. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{6}$.

Fig. 6 The figure exhibits the relation between the collaginous (*cf*) and reticular (*r*) fibers in the region adjoining a nodule of a patch of Peyer in the small intestine of man. Note the interlacing of fibers at the border of the lymphoid tissue. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{6}$.

blacken; one is justified in assuming that this reaction to the Bielschowsky method, with reasonable care, is constant.

B. COLLAGINOUS FIBERS

That the typical reticular fibers of lymphoid tissue and the typical collaginous fibers of dense fibrous tissue take on different colors after the silver impregnation has been generally recognized, at least by the Italian writers, Levi ('06), Ciaccio

('07), Cesa-Bianchi ('08), Balabio ('08), Alagno ('08). The reticular fibers assume a dense opaque black while the collaginous fibers take on a golden brown when well differentiated. Yet if one examines carefully those points at which the two tissues blend one encounters much difficulty in determining whether the black color of the coarser collaginous bundles is due to the opacity of the brown bundles—which are of considerable size and thickness and often of great density—or to the presence within the coarse collaginous bundles of finer, blackened, reticular fibers. In some locations the latter relation is apparent. For example, in the perifollicular plexus about the lymphatic follicles, described by Ciaccio ('07), one finds the characteristic lozenge shaped meshes of the 'reticulum' extending into the adjacent collaginous tissue of the trabecula in lymphoid organs or of the tunica propria and submucosa in the digestive tract, but there the reticular fibers are nearly always clear and sharp among the collaginous fibers of the smaller fibrous bundles (figs. 5, 6, 7, and 13). As the bundles increase in size, however, the difficulty of distinguishing the exact outlines of the two types of fibers increases.

Another difficulty in the way of exact and positive differentiation is the variable result of silver impregnations. With varying degrees of impregnation, reduction, and toning the collaginous fibers may lose their typical golden brown and acquire an increasingly opaque condition. This is specially prone to occur if the sections are overtoned in the gold chlorid bath. One halts, therefore, between the idea of similarity if not positive identity of collaginous fibers and "reticulum" and the opinion of Mall ('01) which regards reticulum as an independent tissue, distinctly differentiated from the collaginous by its somewhat different chemical reactions, a view not fully accepted by Studnicka ('03) nor yet generally adopted by German authors (Fürbringer, '09). Yet if one uses care with the silver process one can obtain from nearly all tissues quite distinctive preparations. Thus in the lung the fibrous tissue of the pleura, as shown by Favaro ('09), as well as that of the "interlobular septa" appears to be formed by golden brown fibers arranged in bundles having the characteristic wavy course together with but few intermingled black reticular fibers, whereas the reticulum in the walls of the alveoli and smaller bronchi, though often composed of coarse typically spiral fibrils, forms an interlacing mass of discreet fibers, or fiber bundles, among which a limited proportion of finer bundles of brownish collaginous fibers may be recognized. In the vascular trabecula of the spleen (fig. 7) the collaginous fibers of the blood-vessels acquire a typical brown while the close network of reticular fibers take on an intense black and have a characteristic, either somewhat regularly spiral, or a reticular course, very different from the irregularly wavy collaginous fibers.

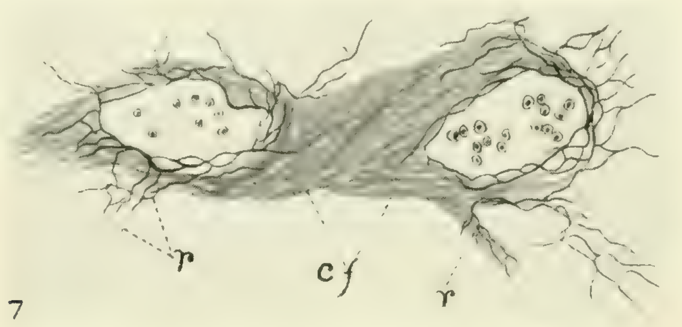


Fig. 7 A vascular trabeculum of a child's spleen. The blackened fibers of reticulum (*r*) show clearly in contrast to the collaginous fibers (*cf*) which in the section are a golden brown. The reticulum surrounds the vessels and is continuous with that of the splenic pulp. Bielschowsky stain. Drawn with Edinger projection apparatus, × 255.

In the trabecula of the lymphatic glands the distribution is not so apparent, the collaginous and the reticular fibers pursuing somewhat similar courses, though the latter are apt to be more distinctly spirillar. From careful examination I am led to believe that the relation simulates, in reverse, that already described (see fig. 2) for the elastic fibers, in that it would appear with considerable certainty in many places that the black reticular fibers are invested or enveloped by a sleeve or coat of collaginous fibrils, so that the latter fibrils consequently assume a spiral course corresponding closely to that of the reticular fibrils. Indications of a similar investment of the reticular fibrils can be found wherever reticulum occurs, but it is not always possible to distinguish with certainty between the collaginous fibers and the protoplasm of mesenchymal or fixed connective tissue cells.

Again in the fibrous perichondrium of hyaline cartilage, as Studnička ('06) has pointed out, there is a considerable layer of blackened fibers marking the border of the cartilage and in the younger types extending into its matrix; the outer layers of the perichondrium are clearly, however, collaginous tissue, and in my preparations present the characteristic golden brown color, sharply distinguished from the intense black of the argentiferous fibers. The matrix of the cartilage in the same sections retains a brownish tint except in the younger specimens and at the margins of the cartilaginous plates in the more mature cases. The blackening of the innermost fibers of the perichondrium which mark the "growing surface" of the cartilage may be explained by the increased affinity for silver shown by the fibers of young connective tissue as compared with the mature, a relation which I am not ready to discuss further at this time.

The remarkable differences in reaction to the impregnation in many of the mature tissues, especially such as contain typical reticulum, would tend to refute the German idea of the identity of collaginous and reticular tissue and to confirm the opinion of Mall that reticular tissue or "reticulum" is a distinct entity, though this latter contention cannot yet be established from the standpoint of the method here used until it is viewed in the light of the histogenesis of the connective tissues, for there the sharp lines of demarcation diminish even to the vanishing point.

Such characteristic differences between reticulum and collaginous fibers as may be observed at almost any point in thin sections of the lymphoid tissues impregnated by silver leave little to be desired in the way of morphological differentiation of these two types of fibers. Such areas are well and accurately shown in fig. 5, from the lymphatic gland of man, and fig. 6 from the margin of a Peyer's patch in the human intestine. One feels, therefore, that the separate and distinct character of collaginous and reticular fibrils in the mature tissues as shown by silver impregnations, fortified as it is by the chemical differences demonstrated by Siegfried and Mall, the one, collaginous, yielding gelatin, the other yielding a "reticulin" presenting different chemical reactions, forms at least a satisfactory working basis for the further study of the distribution of these fibers as shown by the Bielschowsky method, a work already begun by Studnička, Ciaccio, Balabio, Alagna, Favaro, Maresh, Cesa-Bianchi and others.

C. RETICULUM

The careful observation of Bielschowsky preparations also yields valuable data as to the finer structure of reticular tissue and the relation of its fibrils to the "fixed" connective tissue cells.

The coarser fibers of 'reticulum' may be readily seen and, where such fibers come into relation with the "knots" of the reticular net, one can observe these fibrils breaking up into a plexus within, or about, the cells as pointed out by Balabio ('08). Somewhat of this arrangement is indicated in fig. 8, though in other places the fibers appear to enter the cell and end either abruptly or, more frequently, pass through the cell in close proximity if not in contact with its nucleus. The appearance of abrupt ending might if only occasionally observed, be due to the passage of fibers out of the plane of the section, but it occurs far too often so that this certainly is not always the case. The finer fibrils, as well as many of the coarser ones, appear as single fibrils though because of the complete opacity of the impregnation one cannot say that this is actually the case. Certainly the larger fibers distinctly show indications of fibrillation.

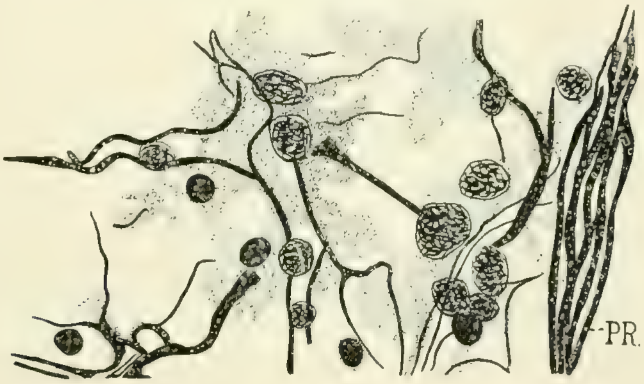


Fig. 8 From a lymphatic gland of man showing the relation of the blackened fibers of reticulum to the branching protoplasm of the fixed connective tissue cells. The small black nuclei are those of lymphocytes. *PR*, perifollicular reticulum. Bielschowsky stain, after stained with acid fuchsin. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom. im.

This leads to the question of the relation of reticular fibrils to the "fixed" connective tissue cells. Are the fibers contained within the cells or are they only in surface contact? It seems to me that Mall has given us the key to the situation with his theory of exoplasmic deposit of the fibrils with constant recession of the endoplasm during development. If one regards reticulum as an immature or least differentiated type of connective tissue it is plain to see that the fibers must readily lie now within the cell or endoplasm, and now without the cell, where they are left "high and dry," as it were, by the complete recession of the endoplasm which leaves in mature collaginous tissue only the nucleated cellular remnants. Since certain fibers, or portions of fibers, would thus lie without the anastomosing syncytial mass of endoplasm while certain others would lie quite as plainly within it we have here a possible harmonization of the otherwise conflicting theories of Kölliker and Bizzozero. The facts of the case as I observe them in silver impregnated sections of embryonal as well as mature tissues appear to coincide with this hypothesis.

Ciaccio ('07) attacked this problem casually in connection with his study of the distribution of reticular tissue in the lymphoid follicles of lymphatic glands and observed a relation of contiguity of fibers and cells, the two being independent. Thus, he says, "le fibrille alla loro volta si diramano in tutti i sense e si montrano independenti dalle cellule."

Balabio ('08), cognizant of the work of Ciaccio, approaches the problem circumspectly, and describes the fibers as "superimposed" upon the cells forming a characteristic close and delicate pericellular plexus. He observed that the cellular prolongations "intertwine among" the fibrils but he was not able to determine "with certainty" whether they were superimposed or whether they "anastomosed in the form of a sort of continuous cellular net." He "limits himself," as he says "to emphasize the fact without pronouncing upon the existence or non-existence of true cellular anastomoses." He is inclined to confirm the theory of Bizzozero for he in one place says "Si puo confirmare conscicurezza quanto gia Bizzozero ed altri affermarono che si tratta di rapporti di sola contiguita."

If one assumes that to disprove the theory of Bizzozero one must find the fibrils at all times outside the anastomosing cells, never within, then proof is not forthcoming. On the other hand proof is also lacking if to demonstrate the theory of Kölliker fibers must always be found within the cellular syncytium. But viewing the tissue in the light of its histogenesis, one need not, as pointed out above, be thus limited in either case, for the fibers of reticulum may, according to this interpretation, come to lie now within, now without the syncytial endoplasm. The examination of such appearances as those shown in any of the figs. 8 to 11, which are accurately drawn with high magnification, or yet more truly if one studies the actual preparations, must convince one at a glance that in mature lymphoid tissue the fibrils of the reticulum are not entirely contained within the fixed cells. The burden of proof lies on the other side.

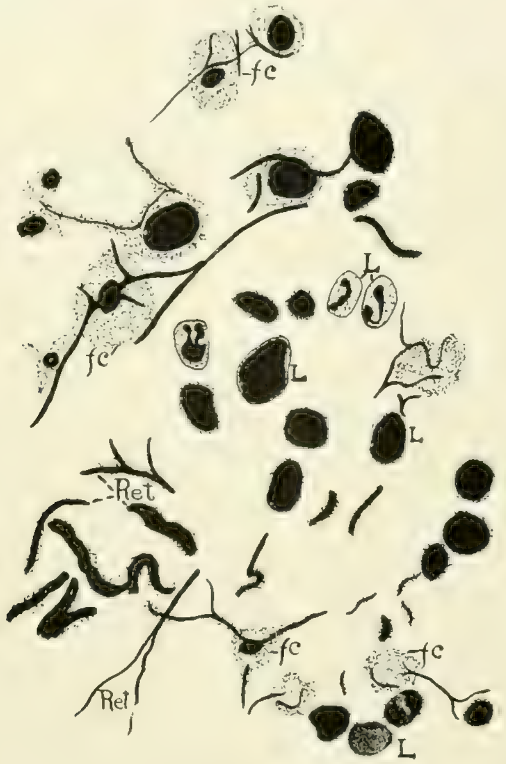
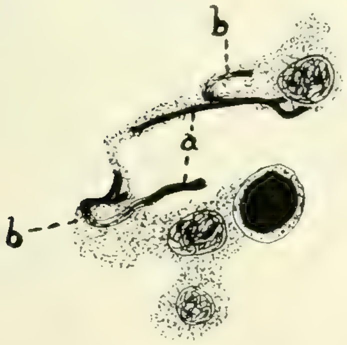


Fig. 9 Reticulum and cells as seen in a thin section through the pulp of the human spleen. No collaginous fibers have been included. Fibers and nuclei are black, the cytoplasm is granular. The fibers are surrounded by a halo of cytoplasm especially distinct wherever their cut ends are directed toward the eye of the observer. *Ret*, reticlular fibers; *fc*, cytoplasm; *L*, lymphocyte. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom im.

The accurate and sharp delineation possible under high magnification between the opaque black fibrils and the light brown protoplasm of the cells presents appearances in thin sections which seem to me to show unmistakably that some portions of the fibrils are certainly contained within the cytoplasm of fixed connective tissue cells. I do not find any such condition in relation to the lymphocytes which are so numerous in the same vicinity. In fig. 10 the fibrils *a* are, in the case of the lower cell at least, certainly outside the cell at one point, viz., where it ends by passing out of the plane of the section. But at the point *b* each fiber makes a distinct loop which can be followed by change of focus. The granular cytoplasm forms a continuous mass but in the midportion of the loop it can be distinctly seen at a level *above* that of the fiber; while at the ends of the loop, in fact at all the solid black portions of the fiber the cytoplasm is distinctly *below* the fiber. It would appear obvious that each fiber has penetrated the cell and must, therefore, during its passage have been found within the cytoplasm. Fig. 10 was drawn from a section of the spleen, but in fig. 11, which is from a lymphatic gland and in which *a* again marks the portion of the fiber above, and *b* that below the cytoplasm, the same condition holds. Such places are extremely abundant, and in thin sections of all the lymphoid tissues examined they can be found with ease, often several in a single field. Again in transections of the coarser fibers, or in oblique sections, the fibers are often seen surrounded on all sides by a light brown halo of cytoplasm. Such appearances are indicated by fig. 9, though it is difficult to depict them accurately even with the aid of the camera lucida because of the extreme fineness of the fibers and the very thin cytoplasmic coat (represented by the stipling) by which they are surrounded. The cut ends of most of the transversely and obliquely cut fibers show the halo of cytoplasm in the actual sections.

The above observations appear to show convincingly that, at least, at times the fibrils lie distinctly within the cells. That they may be so found, as also without the cells, is in harmony with Mall's suggestion as to the ontogenetic relationship of "reticulum" and other connective tissues since he supposes this tissue to represent a less mature type than the collaginous, one in which the primitive relations of endoplasm and exoplasm still persist to a considerable extent. That this is the case is, perhaps, indicated by the fact that in developing mesenchymal tissue one finds fibrils, bearing similar relations to the endoplasm and exoplasm of the connective tissue syncytium, and which like reticulum blacken with the silver impregnation (fig. 4).



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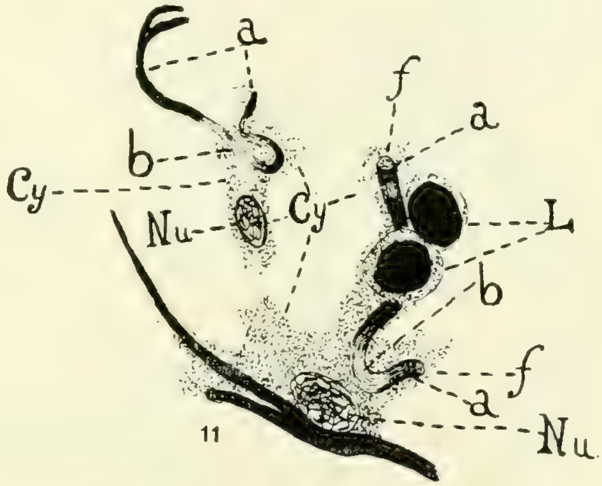

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Fig. 10 Accurately drawn from a section of the spleen of man, showing the actual course of fibrils of blackened reticulum through the cytoplasm of fixed connective tissue cells. The parts *a* of the fibrils end by making a sharp turn which passes out of the plane of the section. The loops formed at *b* are shaded light, and in the section they lie below the level of the cytoplasm as readily demonstrated by change of focus. The black portions, *a*, are above the level of the cytoplasm. Bielschowsky stain. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom. im.

Fig. 11 Areas similar to those shown in fig. 10, but drawn from a section of a lymphatic gland of man. Similar appearances were very numerous in this section. *a* and *b*, as in the preceding figure; *cy*, cytoplasm; *f* cut ends of fibrils; *L*, lymphocytes; *Nu*, nucleus of a fixed connective tissue cell; at the top of the figure a fibril forms a U-shaped loop which passes through the cytoplasm of a "fixed" cell, entering from below and coming out above. Two similar fibers are also shown. Bielschowsky stain, after stained with acid fuchsin. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom. im.

Yet bearing in mind that we are dealing with a method of impregnation only, and are subject to all the limitations of such methods, one is not fully warranted in drawing inferences of chemical similarity between the mesenchymal and the reticular fibrils.

D. FIBROGLIA

The occurrence of fibrils blackening with silver in the mesenchymal cells suggests a possible identity with the fibroglia fibrils of Mallory ('03, '04) for such fibrils were found by that observer to be abundant in developing connective tissue. In order to accurately compare the fibrils shown by these two special methods one must first consider the mature tissues, only thereafter the developing tissues.

In mature tissues Mallory states that fibroglia fibrils "are not very common in normal tissues except possibly in one situation and have to be hunted for with an oil immersion lens." This is certainly not the case with the argentiferous fibers which occur abundantly in a great variety of places among normal tissues and which are of sufficient size to be seen as networks among the other fibers with very low magnification. Again the staining reactions of the two sets of fibrils are different. Mallory describes the basement membranes as the "one situation" where fibroglia fibrils are common in normal tissues, and the subepithelial basket-cells of the sweat glands—regarded by Benda ('93, '94) as muscle cells—as the place where the largest fibroglia fibers occur. As these last fibers can be easily located they form a definite unit for comparison. With Mallory's stain they are red; with iron haematoxylin they blacken when the stain is not too much extracted (fig. 12). Both of these reactions are characteristic for fibroglia, and, as McGill ('08) has shown, they are also characteristic for myoglia fibrils. But with silver impregnation these fibers are not in the least blackened, nor is the thin

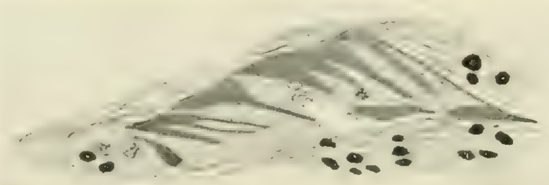
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Fig. 12 The basket-cells of a coil gland of the human finger-tip, darkened by haematoxylin. Haidenhain's iron-haematoxylin. Camera lucida; occ. 1, obj. $\frac{1}{12}$ hom. im.

layer of collaginous tissue upon which they rest. It would therefore appear that the basement membrane of the sweat glands, unlike most other basement membranes, contains no reticulum but is formed by collaginous fibers together with the peculiar basket-cells, be they fibroglia or muscle. If one compares in the same way the reticulum of lymphoid tissues one arrives at similar conclusions as to the non-identity of 'reticulum' (viz. those fibers which blacken with the Bielschowsky method) and fibroglia. It would therefore appear that in the mature tissues there is no identity between fibroglia and reticulum nor for the same reasons can there be between fibroglia and the fibers which blacken with Bielschowsky's stain. These last are identical with certain fibers which are colored blue by Mallory's stain.

SUMMARY

Briefly summing up we find that the Bielschowsky stain applied to the connective tissues of mature individuals exerts a selective action, blackening certain fibers which are certainly not identical with either elastic or fibroglia fibers, which in many cases certainly are identical with the fibers of reticulum, and which in some cases show a certain tendency suggesting possible transitions between reticular and collaginous fibers. The typical collaginous fibers do

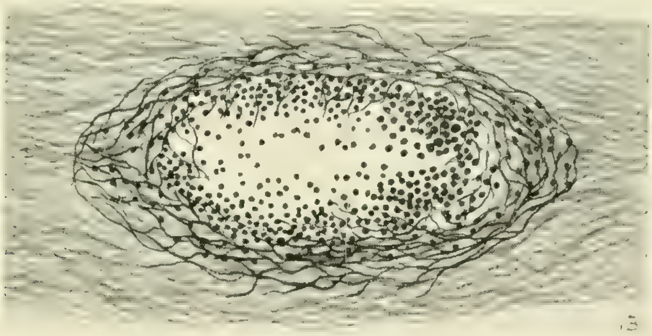

Fig. 13 A small lymphatic nodule from the submucosa of the human esophagus, showing the 'perifollicular plexus' of Ciaccio sharply defined, but with reticular fibers intertwining with the collaginous fibers.

The collaginous tissue is drawn free-hand, the reticulum by camera lucida; occ. 1, obj. $\frac{1}{6}$.

not blacken, but take on a characteristic golden brown color; nevertheless, in certain locations and under certain conditions some fibers which we have been accustomed to regard as collaginous, e.g., within dense connective tissue bundles or in embryonic mesencyhme, do blacken somewhat, though never so typically nor with such clear and sharp definition as do the fibers of "reticulum" in mature tissues. The further elucidation of this atypical reaction of the collaginous fibers must be sought in the histogenesis of the connective tissues. For the present we may safely consider the black reaction of fibers of mature connective tissue to the Bielschowsky stain to be distinctive of "reticulum" in all satisfactory preparations, viz. those in which the collaginous fibers assume a golden brown tint.

When such tissues as nerve, muscle and embryonic mesenchyme are excluded the Bielschowsky method serves as a well-nigh specific stain for the reticulum of Mall.

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STUDIES ON THE PANCREAS OF THE GUINEA PIG

R. R. BENSLEY

From the Hull Anatomical Laboratory, University of Chicago

FIFTEEN FIGURES

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INTRODUCTION

Since the discovery by von Mering and Minkowski in 1890 that complete removal of the pancreas was followed in dogs by a rapidly fatal diabetes, the interest in the pancreas as an organ of internal secretion has been extreme. Laguesse, in 1893, on anatomical grounds, came to the conclusion that the islets of Langerhans were engaged in internal secretion, and Schaefer ('95) suggested that in diabetes lesions would probably be found in these structures. Since that time an immense literature of investigation has grown up in which one might expect to find answers to the perplexing questions which have arisen concerning the relations to one another of the various histological elements which compose the pancreas. It is not the purpose of this paper to give a résumé of this literature, for that has been well done in the recent summary made by Laguesse ('06–'08) in the Revue d'histologie générale. It will suffice here to point out some features in which there are still considerable differences of opinion and to indicate as far as may be possible the reasons of this disagreement.

The differences of interpretation of the islets of Langerhans are well known. To the majority of histologists they are structures which, though originating from the pancreatic anlage in embryonic life, yet maintain a separate existence and full specificity. Others regard them as temporary phases in the history of the acini of the pancreas, denying them any special function, while still others believe that they represent products of change of the acinus cells, performing, however, a special function of internal secretion while in the islet condition.

When we come to consider questions of anatomical fact rather than of interpretation it is more surprising that there should be differences of opinion. It is difficult to understand, for example, why one group of investigators maintain that the islets of Langerhans are anatomically independent of the rest of the pancreatic tissue, and possess a limiting capsule, while another group maintains just as strongly that they are everywhere continuous with acini or ducts.

In the opinion of the writer the reasons for these and other differences of opinion are to be found in part in the lack of adequate technical methods for the investigation of the pancreas, in part, also, in the inadequate definition of the islet cell which is current in the literature. For, although we know, as a result of the observations of Laguesse ('99), Tschassonikow ('00), Mankowski ('01), Lane ('07), and others, not only that the cells of the islet contain granules which are characteristic of them, and which differ in size, refractive power, and solubility from the granules of the zymogenic cell, but also that there are two types of islet cell which differ *inter se* in the nature of their granular contents, yet the prevailing definition of an islet cell is by means of negative characters. Thus an islet cell is defined, not by what it has, but by what it has not. It is not difficult to see how far astray such a definition of an islet cell might lead one, for any cell which was reduced by experiment to a sufficient degree of negativity would become *ipso facto* an islet cell, and thus a wholly false idea of the relations of islet cells to other cells, and of the formation of islet cells would grow up. It was apparently such a definition of islet cells by negative characters that Dale ('05) had in his mind when he described the changes brought about in the pancreas by secretin stimulation as of such a nature as to assimilate all of the cells to those forming the ducts and the centro-acinous cells, and interpreted the cells so modified as islet cells. Similarly Vincent and Thompson ('07) described the conditions found as follows: "There were all transitions to be found between the most strongly granulated of alveolar cells and the clearest of islet cells;" and again: "Transitions as indicated by varying amounts of zymogenous granules in the different cells are frequent." It is not difficult to understand why an investigator who regards the islet cell as something having a definite structure and granules peculiar to itself should differ, both as to his account of the facts, and as to their interpretation, from one who thinks that they are simply cells which contain no zymogen granules and no basophile substance.

It is obvious that before we proceed to study the formation of islet cells from other elements we must know what islet cells are, so as to avoid the error of mistaking for islet cells elements which in reality belong to another category.

These remarks apply with equal force to observations on the effect of certain experimental conditions on the number of islets of Langerhans in the pancreas. For before we can know what this effect is we must be able to diagnose with certainty islet tissue and not confuse it with other tissues. In this connection, also, it may be pointed out that we do not know for a single species, nor have we an adequate method for ascertaining, the total number of islets of Langerhans in the pancreas, or the normal range of variation in this respect. Yet we have numerous records of experimental researches which deal with increase or diminution of the number. For example, Dale and Vincent and Thompson claimed that the number of islets of Langerhans were greatly increased by secretin stimulation and by inanition, while Lewaschew ('86) claimed a similar increase as the result of pilocarpine stimulation. The careful counts of the islets in sections made by Opie ('00), Heiberg ('06), and Laguesse ('08), give us an idea of the relative numbers in different parts of the pancreas, but the results obtained by Laguesse by the more laborious method of reconstruction of single lobules indicates that the real number is probably much in excess of that obtained in such counts. When we apply the method of counting islets in sections to the investigation of the question whether the number of islets has been increased by this or that experimental procedure, we at once introduce a new source of error, for it is obviously impossible to examine and count the islets in a complete series of sections of the whole pancreas, and if we examine only selected portions of the pancreas our results are open to the objection that the part so selected may not be representative even of the region from which it has been taken, for the results of Laguesse and others indicate that there may be wide variations in the relative number of islets even in adjacent pieces of the pancreas.

The differences of opinion that exist with respect to the relation of islets to ducts are not at first sight easy to understand, for it would seem to be an easy matter to demonstrate connections with ducts if such exist. Nevertheless Laguesse maintains that the islets are, in general, in direct continuity with ducts, though the majority of observers are agreed that this is not the case. The apparent reason of this is that few investigators have patiently worked through complete series of sections as Laguesse has, and there was no method for demonstrating the connections when the methods of injection and impregnation failed. There can be, however, no doubt as to Laguesse's results, for his figures show clearly the connections which he describes. The only question is how often the connections with ducts occur. This question can only be answered if we have at our disposal a method which takes the place of the injection method and brings all the ducts of the pancreas to view including those described by Laguesse as going to islets of Langerhans.

The purpose of the investigation, the results of which are recorded in the following pages, has been to find methods for the accurate diagnosis of the several epithelial tissues of the pancreas from one another, and to apply these methods to the questions raised by experimental studies of the pancreas as to the relations existing between the islets of Langerhans on the one hand and the acini and ducts on the other. A second object was to apply methods of accurate enumeration to the islets of Langerhans under different experimental conditions. A third object was the determination of the true anatomical relation of the islets of Langerhans to the ducts of the pancreas.

The work is a continuation of that begun in this laboratory by M. A. Lane, who, following the demonstrations of the specific granules of the islet cell by Tschassonikow ('00), and Laguesse ('99), and that of the presence of two sorts of cells in the islet by Schulze ('00), and Diamare ('99), established criteria for distinguishing the granules of the two sorts of cells from one another and from the zymogen granules of the acinus cells. As far as the pancreas of the guinea pig is concerned the technical needs have been fully met by new methods, and some of these methods may be applied, to what extent will be indicated in a suitable place, to the study of the pancreas of other animals.

TECHNIQUE

1. *Methods for the demonstration of all the islets of the pancreas, permitting enumeration*

a. The neutral red method. The animal is killed by bleeding. A cannula is introduced into the aorta and a solution of neutral red in isotonic salt solution containing one part of neutral red in 15,000 of solution is injected. Immediately the pancreas takes on a rose tint, and after a sufficient depth of color is obtained, which must be learned by experience, the injection is stopped, the pancreas is removed, and examined. If the injection has proceeded a sufficient length of time, a lobule of the pancreas, placed under a cover glass and examined, will show the islets of Langerhans stained an intense yellow red, while the rest of the pancreas will show a faint rose tint. In a short time after the preparation is mounted, owing to reduction of the dye, the acinus tissue is bleached and the islets remain the only stained elements in the pancreas. If the preparation has been overstained, that is, has been stained for too long a time, exclusion of oxygen from the preparation suffices, in a short time, to reduce the excess dye in the acinus tissue, leaving the islets sharply differentiated as before. In such a preparation it is easy, though somewhat laborious, to count the entire content of islets in a pancreas of suitable size, without missing islets and without duplicating counts. The clearness of such a preparation is sufficiently indicated by fig. 1 which shows such a group of lobules simply pressed apart under cover glass without teasing or sectioning. For counting it is necessary to enlist the help of two or more helpers in order to complete the count before the inevitable reduction of the dye overtakes the islets, and begins to cause the disappearance of the smallest of them. With the help of one man mounting preparations, three men can easily count an entire guinea-pig pancreas in one and a half hours, using for this purpose counting machines. The procedure is as follows: The pancreas is divided into minute masses 3 or 4 mm. square and of the thickness of single lobules, which are separated as much as time permits by tearing the connective tissue with forceps. These pieces are mounted in salt solution under cover glasses, without pressure (pressure easily disintegrates the larger islets and disperses their cells among the acini), and counted. The boundaries of the lobules serve as a guide to prevent counting islets twice, and with a little practice a fair degree of accuracy can be attained. After all the islets are counted the pieces of pancreas are again collected from the slides, keeping separate the pieces belonging to the major divisions of the pancreas, and after pressing lightly between layers of filter paper to remove excess salt solution, are placed in weighing tubes and weighed. Thus a total count of the islets, and weights are obtained, which enable one to determine with a fair degree of accuracy the ratio of islets to pancreas, and to body weight.

The chief sources of error in this method are due, first, to overstaining of the pancreas, when the zymogen granules stain slightly, and so obscure the smaller islets, thus making the resting pancreas more difficult to count accurately than the active pancreas where there are few zymogen granules; second, to the high refractive index of the zymogen granules making the pieces less transparent; third, to careless mounting of the pieces, so that they are not sufficiently transparent; and lastly, to the error in comparative weighing, owing to different amounts of salt solution being abstracted from the pieces in different cases. The latter error might be considerable if several different workers were preparing the material for weighing, but in the hands of a single worker following a definite routine, the results should be fairly accurate. The neutral red method has been successful for demonstration of the islets in all pancreases examined, which include the following: Cavia, Lepus, Mus, Felis, Canis, Sus, among mammals; Anolis, Chelydra, among reptilia, Rana, Bufo, and Necturus, among amphibia; and Columba among birds. As a method for enumeration it is most successful in the rodents, less so in the cat and dog, for the reason that in the latter species the zymogen granules take up more of the neutral red, and on the contrary the cells of the islets, containing relatively fewer granules, stain less intensely than those of the rodent species. For these animals the next method is preferable.

b. The janus green method. For this method the same technique is followed as in the preceding neutral red method. A word is necessary here, however, with regard to the dye. It is necessary for this purpose to secure the janus green recommended by Michaelis ('00) for staining intra vitam certain cell granulations. This, Michaelis says, is 'diethylsafraninazodimethylanilin.' The corresponding dimethyl dye is ineffective both for the purpose for which Michaelis recommended janus green and for staining the islets of Langerhans. In this country the correct compound may be obtained from L. A. Metz and Company, New York. Several samples of janus green obtained from agents and bearing G. Grübler's label were worthless for this purpose. The janus green is injected in the form of a 1 in 15,000 solution in isotonic salt solution, or, for dogs and cats, in Ringer's solution. When the whole pancreas has taken on a deep blue green color the injection is complete. The pancreas is then covered up with the intestines, etc., so as to exclude the air. Reduction of the dye then proceeds rapidly and at the end of fifteen or twenty minutes if the pancreas be examined it will be found to have a distinct red color with a tinge of blue. This reduction must be checked at the proper moment, either by exposure of the pancreas to the air, or by the injection into the duct of either ammonium molybdate solution or a solution of potassium iodide. But, for fresh study, it is simply necessary to divide the pancreas into sufficiently small fragments so that oxygen from the air may have free access to all parts. Such a preparation shows the islets deeply blue on a red background. This method is not so suitable for total counts because, as Michaelis has pointed out, the reduction of the dye is accomplished by the splitting off of the dimethylanilin group leaving a safranin which cannot be reoxidized to the original form, as in the case of the neutral red and methylene blue leucobases.

For the cat and dog where the size of the pancreas is so great as to preclude the possibility of a total count within the time limits permitted by the rate of reduction of the dye, the janus green method is preferable under proper precautions to the neutral red method for establishing ratios between the numbers of islets and the weight of the pancreas containing them, with a view to estimating the total number in the pancreas, because the total reduction of the dye in the acinus tissue to a red safranin and the resulting color contrast eliminates for these pancreases the difficulty due to the staining of the zymogen granules.

2. Vital staining methods for demonstrating the ducts and their relation to islets and acini

a. Pyronin method for demonstrating the ducts. Pyronin having relatively less color value than the preceding dyes is used in a higher concentration for vital staining. A 1–1000 solution in isotonic salt solution is employed and injected from the aorta as described in methods 1 and 2. It may be combined with neutral red, janus green, or methylene blue, in the same solution, thus giving, by reason of the different selective affinities of the dyes, double vital stains. The pancreas stained with pyronin alone takes on a light rose tint, and pieces of such a pancreas mounted in salt solution under a cover show intensely red stained duct cells including the whole duct system of the pancreas from the main duct to the last centroacinous cell. The islets stain faintly rose color with pyronin, but the stain is not sufficiently intense to permit of an accurate demonstration of the relations of islets and ducts. For this purpose it is necessary to make a double stain with pyronin and neutral red or with pyronin and janus green. In the former case the islets come out deep yellow red, the ducts rose red, and it is possible in such a preparation to determine accurately in a simple mount in salt solution, which are duct cells, and which are islet cells. In the double stain with pyronin and janus green, however, the contrast is very striking for every islet cell is stained the characteristic slate blue of the janus green, while every duct cell or centroacinus cell is stained the deep rose red of the pyronin. Ganglion cells of the sympathetic ganglia and nerves also stain rose-red in pyronin.

b. The methylene blue method for the demonstration of the ducts. Inject from the aorta with a one in 10,000 solution of methylene blue in isotonic salt solution until the pancreas has a uniform blue tint. Successful preparations are those in which the blue penetrates and stains the pancreas almost instantaneously. When a sufficient depth of color has been obtained the methylene blue is quickly fixed by injection of a 5 per cent solution of ammonium molybdate from the pancreatic duct. If the methylene blue has penetrated slowly, however, it will be found that all the dye in the central portions of the pancreas has been reduced, and it will be necessary in this case to expose the gland to the air before fixing, in order to re-oxidise the leucobase to the blue dye. In either case the preparation may be passed through alcohol to xylol according to the usual Bethe method, and either examined as a preparation in toto, or imbedded in paraffin and sectioned. Much the best results are obtained by the total preparations, which eliminate the sources of error due to the section method, but it is necessary to resort to the section method for the demonstration of the duct relations of those islets of Langerhans which are deeply imbedded in lobules of the pancreas. In these methylene blue preparations, when successful, the only structures deeply stained (excluding of course the nerves) are the cells of the smaller ducts and the centroacinus cells. The stain is complete, in a suitable preparation every duct cell belonging to these categories being stained. To use this method for the demonstration of duct relation to islets in the case of the free islets of the interstitial tissue it is advisable to double stain with a solution containing one part in 15,000 of neutral red and one in 10,000 of methylene blue. In such a preparation ducts going to islets may be expected to be blue while the islets themselves are red.

3. *Combination of vascular injection and vital staining for determining the relation of the vascular system to the islets*

The object of this method is to give an accurate delimitation of the islet tissue in preparations which are injected with a colored mass, for demonstrating the relations of the arteries and veins to the islets. Stain the pancreas by injection from the aorta with janus green as described under method *b*. Allow twenty minutes for reduction of the dye, then follow up with a carmine gelatine solution kept fluid by the addition of 10 per cent of potassium iodide. When the injection is complete inject, by a cannula tied in the pancreatic duct, a 5 per cent solution of ammonium molybdate. This precipitates the gelatine and at the same time fixes the janus green. Pieces of the pancreas are then washed as rapidly as possible with cold water to get rid of the ammonium molybdate, then quickly dehydrated with absolute alcohol, cleared in toluol, and mounted in balsam. During the dehydration the safranin resulting from the reduction of the janus green is extracted, and the resulting preparation shows slate blue islets clearly outlined and defined, but so transparent that with a binocular microscope, one can follow every blood vessel in them. No section cutting is necessary for the pancreas of the guinea pig or that of the rabbit. The latter in particular, on account of the thinness of the lobules, is well adapted for this kind of preparation. Double injections have also been made by this method, but offer no advantages over the injection of a single color-mass.

4. Methods of fixation

a. Lane's methods for demonstration of the A cells of the islets of Langerhans:

A. Fix the tissue for two to four hours in:
Saturated alcoholic solution of mercuric chloride,
Two and one-half per cent solution of potassium bichromate; equal parts.
Wash in 50 per cent alcohol; then pass through graded alcohols to absolute; etc.
Sections 3 micra thick are stained in neutral gentian (see below).

B. Fix the tissue in alcohol 70 per cent. Stain sections in neutral gentian (see below).

b. Lane's method for the demonstration of the B cells of the islets of Langerhans:

Fix in the following solution four to twenty-four hours:

Potassium bichromate	2.5 grams
Mercuric chloride,	5.0 grams
Distilled water	100.0 cc.

Stain in neutral gentian (see below).

c. Formalin bichromate sublimate method:

Fix twenty-four hours in the following solution:

Neutral formalin	10 cc.
Zenker's solution without acetic acid	90 cc.

Stain, with neutral gentian, acid violet-safranin, or acid fuchsin-toluidene blue (see below).

d. Acetic osmic bichromate method:

Fix tissues for twenty-four hours in the following:

Osmic acid 4 per cent	2 cc.
Potassium bichromate, 2.5 per cent	8 cc.
Glacial acetic acid	1 drop

Prepare sections, which must be less than 4 micra in thickness, for staining as follows: The sections fastened to the slide by the water method are freed from paraffin by toluol, then passed through absolute alcohol to water. They are then treated for one minute with a 1 per cent solution of potassium permanganate, then for the same length of time with a 5 per cent solution of oxalic acid, then thoroughly washed in water, after which they may be stained by the acid fuchsin methyl green method, the acid fuchsin toluidene blue method, the neutral gentian method, or the safranin-acid violet method.

In addition to the foregoing solutions the following were occasionally employed: Benda's fluid for mitochondria; Zenker's fluid; Hermann's fluid; Flemming's two mixtures; aqueous mercuric chloride; trichloracetic and trichlorlactic acids.

5. Staining methods

a. The neutral gentian method. Neutral gentian is the name given to the neutral dye obtained when a solution of gentian-violet (crystal violet) is precipitated by its equivalent of a solution of orange-G. If the correct quantity of the orange-G solution be added, practically complete precipitation is obtained, but if excess of the orange-G solution be added, the neutral dye is redissolved. In the latter case it is necessary to add more crystal violet to the solution to secure complete precipitation. The neutral dye thus obtained is practically insoluble in water, but is freely soluble in alcohol or acetone, either of which may be used to make the stock solution. For staining add the stock solution of the neutral compound to 20 per cent alcohol until a solution having the color of a good haemalum solution is obtained. Allow this solution to stand twenty-four hours to permit the excess dye to separate out, when it may be employed for staining as follows:

Stain in neutral gentian solution 24 hours.
Blot between several layers of filter paper.
Dehydrate in acetone.
Place section in toluol.
Differentiate in:

Absolute alcohol	1 part
Oil of cloves	3 parts

Wash with toluol and mount in balsam.

b. The safranin-acid violet method. Prepare the stain by precipitating a saturated solution of safranin O with solution of acid violet. The precipitation should be complete, leaving on subsidence a faintly violet supernatant fluid. Dissolve this precipitate in absolute alcohol. To make the solution for staining, dilute this stock solution with its own volume of distilled water, allow to stand thirty minutes, then filter and use.

The method of using this solution is the same as for neutral gentian except for the fact that staining is from five to thirty minutes.

c. The acid fuchsin methyl green method. This method has much in common with the well-known method of Galeotti but is somewhat simpler.

The staining solutions are:

1. Altmann's acid fuchsin anilin solution:

Acid fuchsin	20 grams
Anilin water	100 cc.

2. 1 per cent solution of methyl green.

The sections after being prepared for the staining process by treatment with permanganate of potassium followed by oxalic acid, are stained for five minutes in the acid fuchsin solution which has been previously warmed to 60° C. Then they are thoroughly washed in distilled water, and dipped for an instant into the solution of methyl green, then washed, rapidly dehydrated in absolute alcohol (alcohols of intermediate strength must be avoided) cleared in toluol, and mounted in balsam. This method has been of exceeding value because in tissues fixed in acetic osmic bichromate it permits of the differentiation of all the epithelial elements of the pancreas, including their granular contents and mitochondria, in a single preparation.

d. The acid fuchsin toluidin blue method. This is the same as the foregoing except that toluidin blue is substituted for methyl green.

e. The neutral gentian, acid fuchsin, picric acid, method. This method has been devised to permit the study of the changes in the mitochondria in a certain common form of degeneration of the pancreatic cell of the guinea pig, which has been described by Mankowski as a form of transition between islet cell and acinus cell. It was necessary in this case to stain the mitochondrial granules and the degeneration granules differentially, which was accomplished as follows:

1. Stain in neutral gentian and differentiate as indicated under 5 *a*.
2. Transfer to acetone, thence to water.
3. Fix the crystal violet stain by a solution of ammonium molybdate acting for five minutes.
4. Wash thoroughly in water, and stain in anilin acid fuchsin (method 5 *c*).
5. Differentiate with a solution of picric acid in 30 per cent alcohol.
6. Dehydrate in absolute alcohol, clear in toluol, and mount in balsam. By this method the Mankowsky granules are stained violet, the mitochondria red.

f. The copper chrome haematoxylin method. This method was employed to stain the mitochondria in the islet cells. Sections fixed in acetic osmic bichromate are prepared for staining by treatment with permanganate of potassium as described above, then treated as follows:

1. Place sections for five minutes in a saturated solution copper acetate in water.
2. Wash in water and transfer to a 0.5 solution of haematoxylin in water.
3. Wash in water and transfer to a 5 per cent aqueous solution of neutral potassium chromate.
4. Wash in water and differentiate in Weigert's borax ferricyanide solution.
5. Wash thoroughly, dehydrate, clear, and mount in balsam.

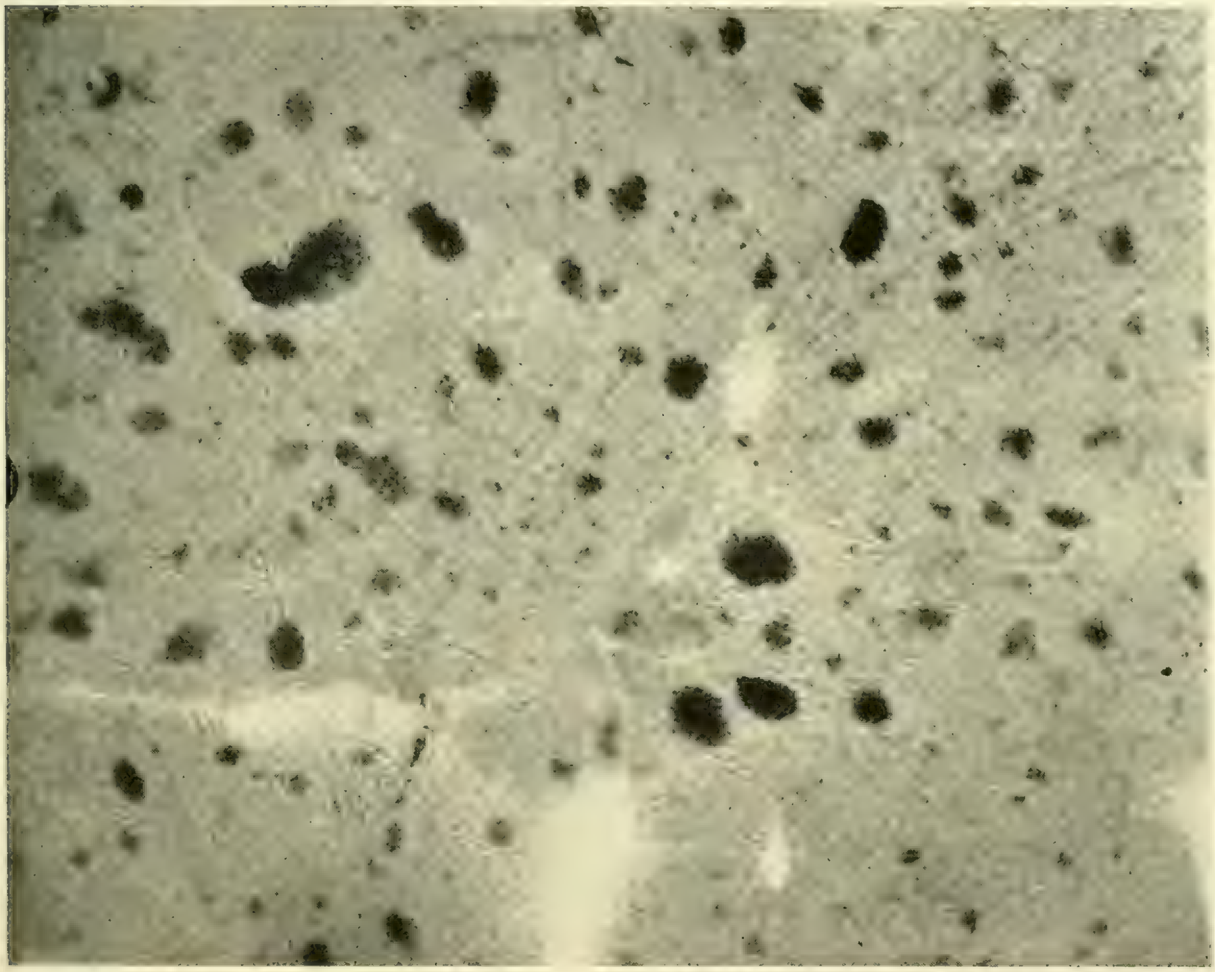
THE NUMBER OF ISLETS OF LANGERHANS IN THE PANCREAS OF THE GUINEA PIG

The determinations of the number of islets in the pancreas of the guinea pig have been made by means of the neutral red vital staining method described under technique. Some idea of the character of the preparations in which the islets were counted may be obtained from figs. 1, 2, and 3, which are reproductions of photographs made from preparations of the pancreas of the guinea pig, stained by injection with neutral red and mounted without teasing and with but slight pressure under a cover glass. All three were made from the same preparation, and so give an idea not only of the large proportion of islet tissue in the pancreas, but also of the variation of this proportion within narrow limits of space. As the figures indicate, every islet from the smallest to the largest stands out clearly stained in the preparations. The largest islet shown in these figures measures 0.5 mm. in diameter, while the smallest measures 50 micra. These are not, however, the limits of size in the guinea pig, for the smallest islet is a single islet cell included among the cells of a ductule or among the zymogenic cells of an acinus, while occasionally, as Dewitt ('06) has pointed out, one may find islets having a diameter of 1 mm. Such single islet cells are, however, comparatively rare in the guinea pig after the first two weeks of life, although in individual cases they may present in considerable number among the epithelial cells of certain branches of the duct system which will be described later.

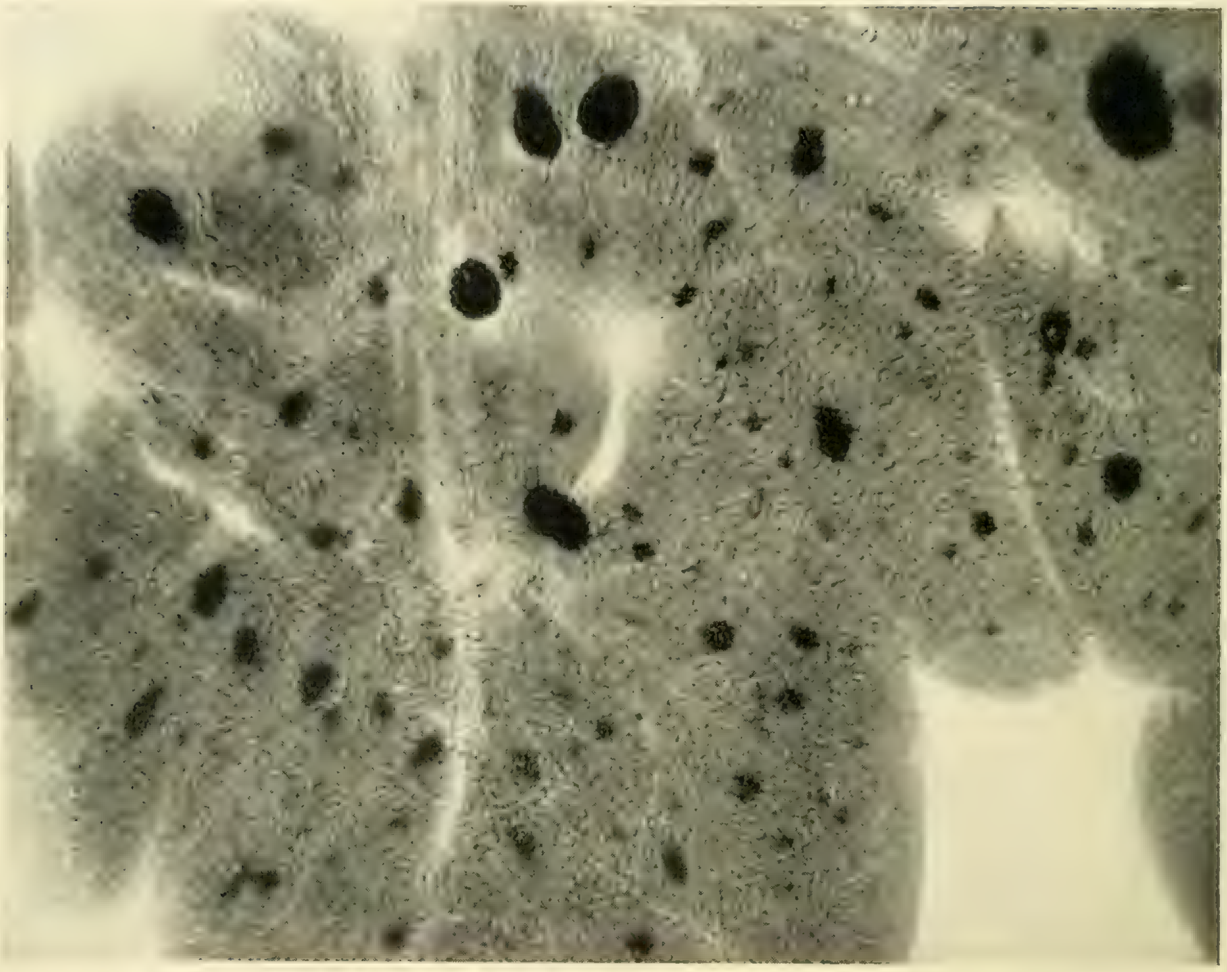
To secure a resting condition of the pancreas the animals were kept without food for twenty-four hours and, in addition, each animal at intervals of twelve hours was given hypodermically two doses of one-twentieth to one-tenth of a grain of atropine sulphate. By this means secretion is checked and the pancreas is permitted to store up zymogen. I did not consider it wise to prolong the period of fasting beyond this limit because of the claim of Dale ('05) and of Vincent and Thompson ('07) that fasting as well as active secretion increases the number of islets. At first I hoped that, when the whole pancreas was considered, the variation in number of the islets would not be great, even though the variation in size was so considerable. As the counts progressed, however, it was speedily found that this hope was not justified, and it became necessary to extend the series far beyond what was originally intended. In the records of the earlier counts only the weight of the animal and the total count of islets were recorded. Later, records were kept of sex, pregnancy, total weight of pancreas, and of the weights and corresponding counts of islets in the different portions of the pancreas. The total number of guinea pigs in which total counts of the islets have been made is ninety-nine. Of these sixty-five were normal resting pancreases, twenty-four were from secretin experiments, six from inanition experiments, and four pilocarpine experiments. In view of the fact that the counts offer no support for the claims of Dale ('05) and of Vincent and Thompson ('07) that the number of islets is increased by secretin stimulation and by starvation, all of the counts might well be included in a single table to show the normal range of variation in the guinea pig. I have, however, decided to keep the series separate and offer as normal resting counts only those cases where the animal had been kept without food for twenty-four hours, and in which the secretion had been restrained by atropine.

It might be supposed that the great differences in total number of islets in different guinea pigs of the same weight would be due rather to the difference in size of the islets than to a real difference in the relative amount of islet tissue present. We have not yet made computations of the relative weights of zymogenic tissue and islet tissue, but it would be quite possible to do this with the neutral red method, and it is hoped that an opportunity will soon be found. It is, however, not the rule that the variation in numbers is compensated by corresponding variations in size, that is, that, where the numbers are large, the islets are relatively smaller than in the pancreas from an animal of the same weight having a smaller number. On the contrary high numbers are often associated with a proportionally high content of islet tissue.

It is, of course, inevitable that the error in such counts would be considerable, owing to the causes to which I have previously referred. Comparative counts made by two different individuals



Figs. 1–3 Photomicrographs of preparations of the pancreas of the guinea pig, made by injection of neutral red into the blood vessels, showing the character of the preparations in which the counts of islets of Langerhans were made, and also the variations in size and frequency of the islets. × 38.



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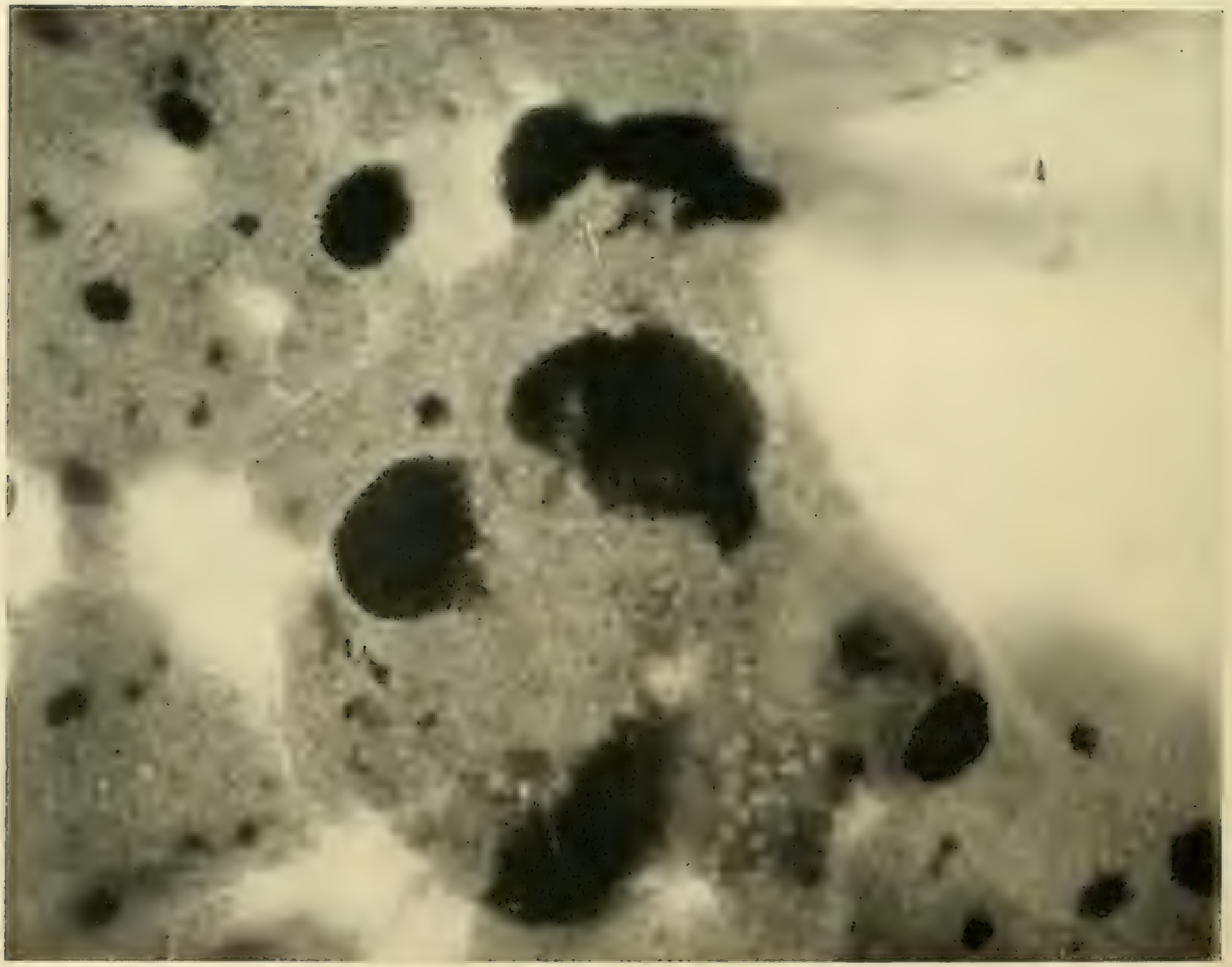

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of the same pieces of pancreas, and recounts of pieces which have been teased a second time and remounted give variations in the case of the resting pancreas of as high as 10 per cent. In the discharged pancreas, on the other hand, where the error due to the concealment of the smallest islets by the zymogen is eliminated, the error is rarely more than five per cent. In making the counts, overstained and understained pancreases were rejected. A certain degree of overstaining, however, is soon corrected after the pieces are mounted under a cover, by the rapid reduction of the dye in the acini. As table 2 indicates, in the pancreas of the new born the islets are so abundant (as high as 338 in a single milligram of pancreatic tissue), that accuracy is not so easily obtained as in the older animals where the number in a milligram of tissue rarely exceeds thirty-five. In these young pancreases the islets are so close together that it is difficult to be sure either of not missing large numbers or of not counting large numbers of them twice. Doubtless these two errors compensate one another to a certain extent, but nevertheless it is probable that the error of counting is greater in these cases than in the pancreas of an older animal.

The enormous variation in the number of islets at all ages makes it difficult to interpret the facts with regard to such questions as whether the number of islets increases with age. In the whole series there were only three animals in which the number of islets was greater than in a guinea pig two days old and weighing 74 grams. There are, however, certain facts which indicate that during the first two weeks of life there is a reduction in the actual numbers of islets, and that thereafter there is a slow production of new islets. These facts will be discussed more fully elsewhere in this paper, but it may be mentioned here that, in the new born guinea pig, in addition to the islets consisting of two or more cells, there are myriads of single islet cells located in the acini and forming a part of the regular row of epithelium in these acini, which do not enter into the counts because with the low powers of the microscope necessary for counting they cannot be distinguished from connective tissue cells which contain large irregular granules stained with neutral red. In the guinea pig one week old, and at all times thereafter, such cells are rare although a few

TABLE 1

Total counts of islets of Langerhans in the resting pancreas of the guinea pig. (This series includes the controls of the experimental series)

WEIGHT IN GRAMS	SEX	TOTAL NUMBER OF ISLETS	PER GRAM WEIGHT OF ANIMAL	WEIGHT OF PANCREAS IN GRAMS	AVERAGE NUMBER PER MILLIGRAM PANCREAS
55		21,365	338.4	0.113	189.07
74		40,842	551.9	0.243	168.0
85		28,912	340.1		
95	female	24,477	247.2	0.138	177.3
104	male	25,862	248.67	0.245	105.5
168	male	18,996	113.08	0.648	29.31
182	female	20,348	111.7	0.707	28.78
204	male	18,916	92.72	0.795	23.83
220	male	23,498	106.8	1.01	23.25
243	female	22,627	93.11	0.682	33.32
245	female	23,972	97.87	0.715	33.52
246	male	19,034	77.82	0.722	26.36
280	female	28,024	100.08	0.859	32.5
304	male	22,984	75.4	1.118	20.5
331	female	25,535	76.5	0.949	26.68
367	male	32,044	87.2	0.939	34.1
465	female pregnant	30,181	64.9		
469		18,302	39.0		
480		22,271	46.39		
485	male	21,974	45.5		
487		25,583	52.7		
490	male	24,641	50.28		
492	female pregnant	32,397	65.7		
494	female	32,374	65.5		
500	male	29,558	59.11	1.178	25.09
500		15,501	31.0		
502	female pregnant	19,029	37.9	1.395	13.71
502	male	18,893	37.6	1.079	17.6
504	male	21,596	42.8		
507	male	37,712	74.3	1.399	26.9
510		24,664	48.3		
520	male	18,094	34.7	1.383	13.08
525	male	22,234	42.3		
525	male	21,908	41.71	1.032	21.2
530	male	33,827	63.8	1.451	23.3
535	male	32,213	62.0		
537	male	27,472	51.1		
540	male	22,925	42.45	1.506	15.22
540	female pregnant	30,034	55.6	1.202	24.98
545	female	24,218	44.4		

TABLE 1—CONTINUED

Total counts of islets of Langerhans in the resting pancreas of the guinea pig. (This series includes the controls of the experimental series.)

WEIGHT IN GRAMS	SEX	TOTAL NUMBER OF ISLETS	PER GRAM WEIGHT OF ANIMAL	WEIGHT OF PANCREAS IN GRAMS	AVERAGE NUMBER PER MILLIGRAM PANCREAS
549	male	21,851	39.7	1.507	14.5
549	female pregnant	32,109	58.4		
565		14,552	22.2		
574	female pregnant	25,620	44.6		
574		14,040	24.4		
580		15,535	26.7		
585	female pregnant	13,318	22.4		
590		17,044	28.8		
607	male	21,558	35.5	2.099	10.27
617	female pregnant	26,716	43.29	1.039	25.7
623	male	26,852	43.1		
628	male	33,016	52.57		
642		22,879	35.6		
647	male	25,320	39.1		
652		39,215	60.1		
658	male	23,438	35.6	1.402	16.7
665	female pregnant	36,902	55.4	1.470	24.4
672	male	31,957	47.2	1.232	25.1
680		26,540	39.0		
689		46,089	66.8		
734		32,551	42.9		
985	male	41,453	42.0		
1029	male	56,722	54.1	2.223	25.5
1150	male	25,722	22.36	2.702	9.5

occur, even in the oldest. Accordingly they must either have disappeared, or have been transformed into cells of another type or have been converted by mitosis or by accretion of new elements into larger islets. The latter possibility is excluded by the fact that the counts which become more and more accurate as the pancreas increases in size show no increase in numbers in proportion to the single islet cells of the pancreas of the new born. Either of the other possibilities involves disappearance of islet tissue.

The evidence that new islets are being produced from time to time will be given in detail in connection with the discussion of the relation of the islets to the ducts. Here it will suffice to state that in accordance with the observations of Helly ('05), Weichselbaum and Kyrle ('09) for the embryonic pancreas and for early life, islets in all stages of formation may be found in connection with the ducts of the pancreas of the guinea pig throughout life.

The most striking feature of table 1 is the great difference between the actual number of islets and that determined by previous investigators by the method of counting in sections. Dewitt ('06) estimated the number of islets in the pancreas of the guinea pig as 1.14 per cubic millimeter. If we multiply the number per milligram shown in my tables, by the specific gravity of the pancreas we obtain the real number of islets per cubic millimeter. The minimum content found by this method is 9.9 per cubic millimeter, the maximum 197.9 per cubic millimeter. The average content of the whole pancreas for guinea pigs between 300 grams and 600 grams weight is 22.28 islets per cubic millimeter, which is 19.5 times the number estimated by Dewitt.

The reason for this discrepancy is obvious enough when one compares the neutral red preparations with sections of the pancreas. In the former every islet stands out clearly defined whatever may be its size, but in the latter many of the small islets require study under oil immersion lenses to distinguish them from accumulations of centroacinous cells or small collapsed ducts, for it is only under the high power that the specific contents of the islet cell can be well seen. Accordingly by the section method of enumeration, practically all of the smaller islets escape the count.

The difference between the real number of islets and the apparent number of them as seen under the low power in sections acquires an important significance when the latter method is applied to experimental investigation. For what reliance can we place on a method of investigation in which the minimum error is 88 per cent of the real number, and the average error 94 per cent?

Table 2 shows the distribution of the islets of Langerhans in the pancreas of guinea pigs in terms of the number of islets in 1 mg. of pancreas. The results are obtained by counting the pieces separately, and then weighing them as described under

Technique. The sources of error in this method of estimation of the ratio of number of islets to weigh are, in addition to those which influence the accuracy of the count, loss of weight or increase of weight owing to the salt solution being hypotonic or hypertonic, loss of weight from the solution of cell proteins, and inequalities from the comparative standpoint in the amount of water abstracted in drying. The question as to the extent of these errors I have tested by comparative weighing of pancreases of guinea pigs of the same litter in the same physiological condition, and also by comparing the ratios of the weights of several pieces of the same pancreas with the ratios of the weights of several pieces of the same pancreas with the ratios of the dried weights of the same pieces. Of the two male guinea pigs weighing respectively 302 and 304 grams and aged forty days, one was injected with neutral red, counted, and the pieces of pancreas collected and weighed, the other's pancreas was freed from fat and weighed without injection. The total weights of the pancreas were respectively 1.118 grams and 1.104 grams, the heavier being from the injected guinea pig. Similarly two new born guinea pigs weighing 55 and 57 grams respectively gave the weights 0.113 and 0.110 for the pancreases, the heavier being again the injected pancreas. As tested by the comparison of the ratio of dried weights to one another with the ratio of the corresponding wet weights of the same pieces, the greatest difference found was 13 per cent.

In table 2 the splenic portion included the omental portion of the pancreas cut off flush with the dorsal posterior extremity of the spleen, the duodenal portion included that part of the pancreas on the right of a line from the superior mesenteric vessels to the pylorus, the body included the rest of the pancreas between the other two.

In this series of forty-six guinea pigs the splenic portion shows the highest average content of islets in 39, and is second in 7. The body of the pancreas is highest in 7, second in 29, and third in 10. The duodenal portion is first in none, second in 10, and third in 36. Thus the condition found in the human pancreas by Opie ('00) is found to hold true for the guinea pig.

TABLE 2

Distribution of islets of Langerhans in the pancreas of the guinea pig expressed as the number of islets per milligram of pancreas

WEIGHT	SPLENIC PORTION	BODY	DUODENAL PORTION
55	201.08	338.07	106.4
74	190.55	160.97	155.62
95	215.10	147.50	132.40
104	120.37	93.6	96.5
182	34.52	24.6	23.83
186	33.22	28.87	19.66
220	24.1	25.4	19.01
245	34.65	35.69	24.83
280	42.03	26.9	22.97
304	21.1	23.7	13.2
327	35.3	33.4	23.2
331	28.81	26.74	22.8
367	38.93	32.9	26.4
495 S*	34.4	19.0	32.1
500	29.1	23.7	20.98
507 S*	22.9	16.53	11.9
507	29.5	27.1	20.07
514 S*	22.7	13.0	10.7
515 S*	16.5	18.5	12.9
520 S*	22.6	15.9	15.2
520	14.93	12.48	9.26
525 S*	30.8	15.7	13.69
525	23.8	15.4	15.8
530	26.2	23.6	16.7
540	16.97	12.77	14.62
540	30.41	25.8	15.8
549	15.24	11.21	13.77
560 S*	30.7	22.7	27.0
575 P°	35.5	28.0	21.0
580 S*	31.7	30.02	19.47
607	11.95	8.49	9.91
610 P°	21.7	14.9	13.5
612 S*	30.25	19.27	19.12
617	29.90	19.67	27.47
620 S*	26.2	18.0	17.0
642 S*	22.73	18.38	16.50
645 S*	21.4	17.03	14.91
647	31.3	22.4	17.0
655 S*	29.3	19.3	20.0
658	19.8	15.4	12.3

* Secretin experiments. °Pilocarpine experiments.

TABLE 2—Continued

Distribution of islets of Langerhans in the pancreas of the guinea pig expressed as the number of islets per milligram of pancreas

WEIGHT	SPLENIC PORTION	BODY	DUODENAL PORTION
665	30.6	19.2	21.9
672	27.03	28.97	18.91
673 S*	25.2	19.0	15.8
845	23.8	15.7	12.3
1029	25.9	27.24	21.88
1150	10.05	7.95	9.46

*Secretin experiments.

Table 3 indicates the range of variation in islet content exhibited by neighboring pieces of the pancreas. The determinations were made in order to test the validity of the assumption which is made in experimental work on the islets of Langerhans that a sample taken from the corresponding portions of several pancreases represents the proportional islet content of those pancreases. In order to do this a small portion of the splenic end of a pancreas which was injected with neutral red was divided into suitable portions, and the total islet content and weight of each portion was determined. The result shows that the islet content of adjacent parts of the same pancreas may vary within wide limits and accordingly that islet estimates made on this basis are not a trustworthy indication of the real islet content of the pancreas.

THE EFFECT OF CERTAIN EXPERIMENTAL METHODS ON THE NUMBERS OF THE ISLETS OF LANGERHANS IN THE PANCREAS

Table 1, showing the great variation in total number of islets for different ages and weights and for the same weights, table 2, showing the variations in the distribution of islets in different parts of the pancreas, and table 3, showing the inequalities in numbers for equal weights of pancreas from the same portion, taken together, illustrate very well the pitfalls that await the experimental investigator who seeks to determine what effect a certain procedure has upon the number of islets in the pancreas. To determine the direction of the change if any, and to estimate its

TABLE 3

Showing variation in number of islets per milligram in neighboring pieces of the splenic portion of the pancreas of two guinea pigs

No. 1

WEIGHT	NUMBER	RATIO OF NUMBER TO WEIGHT
0.018	598	33.2
0.028	1092	39.0
0.046	1054	22.9
0.030	1136	37.8
0.040	1874	46.8
0.039	1151	29.6
0.028	935	33.3
0.052	1237	23.7
0.029	1261	43.4

Total weight of splenic portion including the above weights	0.479
Total number of islets in the splenic portion	16,606.0
Average number of islets per milligram	35.5

No. 2

WEIGHT	NUMBER	RATIO OF NUMBER TO WEIGHT
0.026	714	27.4
0.025	605	24.2
0.030	882	29.4
0.025	731	29.2

Total weight of splenic portion	0.494
Total number islets in splenic portion	10,744.0
Average number of islets per milligram	21.7

amount by the method usually employed of selecting a sample of the pancreas from the splenic end and comparing the number of islets in a certain area of section is untrustworthy, unless a very large number of sections be examined, and even then the results may lead to incorrect conclusions owing to the unequal distribution of the islets in the pancreas and to the difficulty of recognizing the smaller islets. Thus, in employing the method of comparison of sections, the investigator makes certain assumptions which reference to the tables will show not to be supported by the facts. In the first place he assumes that pieces taken from the corresponding portions of the pancreas of different individuals are in the same degree representative of the total contents of islets in the pancreas. Table 2 shows that this is not the case for the guinea pig. In the second place he assumes that such pieces, carefully selected, are to the same degree representative of the portion of the pancreas from which they are taken. Table 3 shows that this may or may not be true. Finally, a very large series of control normal animals must be taken to insure that the worker has an adequate appreciation of the range of individual variation. Another factor which vitiates the result of the section method is the fact that the islets are rarely located near the surface of the lobules, so that a section through the surface layers of the lobule may show few islets and small islets when the lobule in reality contains many and large ones.

In view of these considerations it seemed desirable to test by the method of total counts the claim of Statkewitsch ('94), Dale ('05), Laguesse ('10) and Vincent and Thompson ('07) that the number of islets and their size is increased by inanition, and that of Dale ('05) and of Vincent and Thompson ('07) that a similar increase is produced by secretin stimulation. It is extremely important that a correct answer should be obtained to these questions, not only because they affect our views concerning the interrelation of the islets and acini in the pancreas, but also because they affect all conclusions as to functional differentiation which are based on anatomical grounds. For, if it can be shown that, as a result of a few hours stimulation with secretin, a pancreas transforms a great quantity, or indeed any, of its acinus tissue into islet tissue, the islet tissue thereby loses all significance it may have as a special organ of internal secretion, and similarly, doubt is thrown on all conclusions as to difference of function, based on anatomical facts of the same order as the differences between the islet cells and acinus cells. It should be pointed out here that the balance theory of Laguesse does not carry the same implications, for it presupposes a special function for the islets of Langerhans and agrees with the ideas of Dale, Vincent and Thompson only to the extent that it assumes that the two tissues may be transformed one into the other in either direction so as to maintain a balance between the functional demands and the relative amounts of the tissue serving them. On the other hand the claims of Dale and Vincent and Thompson that islets are formed from acinus tissue in a brief period of time as a result of secretin stimulation or of inanition, admit of no such functional interpretation, nor indeed of any, but that the islet tissue is simply a negative phase of the acinus tissue, notwithstanding the fact that the authors in question express themselves with some reserve on this topic. The confirmation of the results of Dale, Vincent and Thompson would support Laguesse's contentions only to the extent that it would show that acinus cells and islet cells are reciprocally potent, the disproving of these results on the other hand would affect Laguesse's hypothesis neither one way nor the other, for it would be simply a negative answer to the question of respective potencies of the two epithelia and would still leave the field open to a positive result by different experimental methods. It is conceivable that under usual conditions of life a fair degree of permanency of islet tissue may be maintained, while under other conditions a sudden functional demand for a greater relative amount of one or the other tissue may result in the transformation of one type of cell into the other. The task of the experimenter is to discover the conditions which will call forth such a response, and as long as his results are negative he is not justified in drawing any conclusion, except such as refers to the direct result of the particular experiment. Thus, complete specificity of islet and acinus inter se can not be proven experimentally, while at least two roads are open for the experimental proof of equipotency. The first method is to establish a graded series of transition types of cells connecting the acinus cell at one end with the islet cell at the other. This would, however, give us no clue as to the direction of the change. It would show that one type of cell could be changed into the other type without proving that the direction of the change was in one direction to the exclusion of the other, or that the change in both directions was possible. The second method is to show that it is possible to cause a considerable increase in one or the other tissue in a short period of time without cell division, by experimental methods, and that there is a corresponding return to the normal after the experimental cause of the increase is withdrawn. Both of these methods have been employed by Dale and by Vincent and Thompson with positive results. Of the value of these results it is impossible without further experimentation to judge, nor is it possible with the data given to repeat the experiments, for the authors in question neither give protocols of their experiments nor any indications as to what may be their conception of the normal range of variation in the number of islets in the pancreas of any of the species examined. Accordingly it has been my purpose to test the question as to the possibility of influencing the number of islets in the pancreas by secretin stimulation, and inanition, by methods less open to critical objection than these employed by Dale, Vincent and Thompson, though I have been unavoidably ignorant of the exact methods employed and the precautions observed by these authors.

1. *Effect of secretin stimulation on the number of islets of Langerhans in the pancreas of the guinea pig*

In beginning experiments to determine the effect of secretin stimulation on the number of islets of Langerhans in the pancreas of the guinea pig it was necessary first to determine that the pancreas of the guinea pig responded sufficiently to secretin injections to make it a good experimental animal. It will be recalled that Bayliss and Starling ('03) showed that the pancreas of the rabbit secreted slowly but continuously, and that the rate of secretion was only slightly accelerated by the intravenous injection of secretin. It might for this reason be urged that the guinea pig, being also a herbivorous animal, would exhibit the same characters, and that therefore the pancreas might be expected constantly to show the high islet content which, according to Dale, Vincent and Thompson goes with a high degree of secretory activity. In my counts of normal animals and experimental controls I have been careful to guard against this objection by securing a cessation of secretion by means of heavy doses of atropine sulphate. In the secretin animals, however, it was not considered desirable to bring the pancreas absolutely to rest by this method before injecting secretin, because it might affect the action of the secretin itself. Moreover, if the claims were true that activity increased the number of islets, this effect should be exaggerated by using for stimulation animals in which a slow continuous secretion had been going on, and in which the initial number of islets might on this basis be expected to be high.

In order to test the question of continuous secretion in the guinea pig it was found necessary not only to withdraw food but to isolate the animals in cages having a grating some distance above the floor, for guinea pigs readily eat their own faeces, and so keep the stomach partly filled, and they will secure this sort of food even on a grating if other animals are with them. Animals kept thus without food, however, show the stomach practically empty at the end of twenty-four hours. In these animals, if a cannula be introduced into the duct, a very slow pancreatic secretion manifests itself, but the pancreas quickly responds to dog secretin introduced intravenously, or subcutaneously.

Bayliss and Starling ('03) have shown that in addition to the intravenous method of administering secretin the latter is active if introduced subcutaneously or into one of the serous cavities. The response in the latter case was, however, much less than with the intravenous method. In experimenting on guinea pigs I tried for this reason, at first, to use the intravenous method, but did not succeed in keeping a single animal alive under stimulation for a sufficient number of hours to justify the expectation that a condition of exhaustion sufficient to test Dale's claims had been produced. I was therefore compelled to resort to the subcutaneous method, which had the advantage of permitting a much larger dosage with secretin. For this purpose secretin made from dog intestine according to the directions of Bayliss and Starling ('02) was injected in doses of 5 cc. under the skin of the belly at intervals of one hour, it having been previously determined that the secretion called forth by such a dose lasted for this period of time. The smaller effect of the secretin given in this way is compensated by the much larger dosage which is possible. In this way the pancreas of a guinea pig after a period of eight to ten hours discharges its zymogen so completely that only a few cells show any zymogen granules. Indeed, I have uniformly secured by the subcutaneous method in guinea pigs a more complete discharge of the zymogen granules than I have ever been able to secure in dogs by the intravenous method associated with frequent withdrawals of blood recommended by Dale ('05).

The secretin for these experiments was obtained from fasting dogs by a uniform technique, using 2 cc. of 0.4 per cent. solution of hydrochloric acid to extract each gram weight of scraped mucous membrane. The solution was filtered and kept slightly acid, and rendered faintly alkaline at the time it was injected. Fresh secretin solution was employed for each day's experiments.

The discharged pancreases obtained by long stimulation with secretin are very favorable for total counts by the neutral red method. The blood vessels are dilated and the pancreas injects well and quickly, and overstaining or incomplete staining is rare. The islets stain as strongly as they do in the resting pancreas, the granule content of their cells being apparently unaffected by secretin. Furthermore, the absence of zymogen granules makes the lobules unusually transparent, enabling one to see even the smallest islets with ease, and so permitting accurate counting.

The first series of experiments was conducted without carrying parallel controls, because I thought that if the increase of islets were so great as to be easily apparent to one using the haphazard section method of estimation, the total number after exhaustion should so far exceed the maximum of the normal counts as to leave no doubt of the issue. This first series, however, while showing uniformly high counts in the secretin experiments, were yet all well below the maximum for resting pancreases of animals of the same range of weight. Furthermore, while the counts tended to be favorable to Dale's claims the islets were just as sharply defined from the acinus tissue as in the normal animals which had been previously counted. Around the margins of the islets there were no cells showing only a few islet granules. All cells showing granules at all were packed full of them and the other cells, acinus, centroacinary and duct cells contained no islet gran-

TABLE 4

Secretin experiments

WEIGHT	NUMBER OF ISLETS	ISLETS PER GRAM WEIGHT OF ANIMAL	TIME OF STIMULATION IN HOURS
Series 1			
589	38,817	65.9	8
590	31,605	53.5	7
590	37,879	64.2	7
618	32,285	52.2	5½
649	36,787	56.6	7
654	31,640	46.6	7
667	31,600	47.3	7
Series 2			
485	21,577	41.4	8
495	26,746	51.73	9
507	22,264	44.9	11
525	29,761	56.5	11
*Controls**			
500	29,558	59.11	
540	30,034	55.6	

* In two controls of this series the staining failed.

Series 3			
620	29,830	48.1	10½
645	26,841	41.61	8½
Controls			
647	25,320	39.1	
672	31,957	47.2	

TABLE 4—Continued

Secretin experiments

Series 4

WEIGHT	NUMBER OF ISLETS	ISLETS PER GRAM WEIGHT OF ANIMAL	TIME OF STIMULATION IN HOURS
655	31,289	47.7	10
673	28,708	42.6	12½

Controls

658	23,438	35.6	
665	36,902	55.4	

Series 5

574	25,441	38.07	12
580	33,329	57.4	10
612	32,599	53.26	6

*Controls**

617	26,716	43.29	
628	33,016	52.57	

*In one control animal of this series the staining failed.

Series 6

505	33,860	66.6	13
514	18,333	35.6	8
515	16,643	32.3	16

Controls

502	18,893	37.6	
507	37,712	74.3	
525	21,908	41.71	

ules. Thus while the counts were favorable to Dale's claims, the examination of the tissue under apochromatic immersion lenses showed no transition forms. Accordingly, I abandoned the method of securing guinea pigs for experiment by purchase from different breeders, and postponed further secretin experiments until I could have at my disposal animals bred and reared in the laboratory and so subjected to the same conditions through their whole life. The animals so reared showed a narrower range of variation in numbers of the islets than those previously counted, and the high and low counts were about evenly distributed among the secretin guinea pigs and the controls. Then, to confirm the suspicion that the previous result had been due to the mixed sources from which the guinea pigs had been obtained I made a final experiment with six guinea pigs obtained from sources outside the laboratory. The counts of these animals (series 6) again showed the wide range of variation previously observed but the results were an unequivocal refutation of the claim that secretin stimulation increased the number of islets of Langerhans, because the maximum number was found in one of the control animals and the minimum in one of the secretin animals. Furthermore, two of the secretin animals of this series approached very closely the minimum counts for the whole ninety-nine normal and experimental animals.

2. *The effect of secretin stimulation on the pancreas of the toad*

In his studies on the effect of secretory activity on the pancreas, particularly in reference to the number and size of the islets of Langerhans, Dale made experiments on the toad, in which, by injection of secretin solution into the dorsal lymph sac, it was possible to bring the pancreas to a state of exhaustion, and keep it in this condition for several days. Eleven toads were so treated, and the result according to Dale were the same in all cases, though it varied somewhat in degree. He says:

> There was a very great increase of the tissue, which we have now frequently described, and have called islet tissue. In the specimen, of which a section is reproduced in fig. 11 the change is very extensive. The exhaustion is very complete, no zymogen granules being found in

any part of the section, and a very large proportion of the whole tissue of the gland has undergone the change into islet tissue. . . . Scattered in this transitional tissue are several fully formed islets of the resting type which were presumably present before the injection of secretin.

It was important to test this conclusion of Dale for if it is possible to so transform a large proportion of the tissue of the pancreas of the toad into true islet tissue, it is hardly worth while expending the energy required to count the islets under experimental conditions in mammals. That transformation of pancreatic tissue into something different was accomplished in Dale's experiments is sufficiently obvious from an examination of his figures, but that the tissue so transformed was really islet tissue is not so clear, for Dale admits that the true islets could be recognized in the midst of the transforming tissue. What then are the clear cells of his preparations? If they are islet cells they should have the normal content of islet cells.

I have found in confirmation of Dale that the toad is well adapted to this type of experimentation for it is very responsive to secretin solution introduced into the dorsal lymph sac. Indeed, if the dorsal lymph sac of a toad is filled with an active secretin solution in the evening, the pancreas will be found next morning so exhausted that the zymogen is reduced to a row of very small granules along the dilated lumen of the acinus. By repeated injections the pancreas may be quite deprived of its zymogen and kept so for several days. If the precaution is taken not to overdistend the lymph sac and to make the injections at frequent intervals, the toad may be kept for as long as seven days with the pancreas in a state of continual exhaustion. Under these conditions, surely, one might expect that the maximum effect of over-secretion would be found.

The pancreas of the toad, moreover, is well adapted for investigation by the methods of staining the islets of Langerhans by means of neutral red, or janus green. The results obtained here are exactly comparable to those obtained in the mammal, the small granules with which the islet cells are studded taking up the red or the green as the case may be, and permitting an estimate of the total content of islet tissue.

The granules may be easily demonstrated in the islet cells of the toad by appropriate methods of fixation and staining. The islets of the toad, like those of mammals, are composed of two types of cells which present similar characters to the corresponding cells of the islets of the guinea pig. The *B* cells, which are the most abundant, show their granules best when fixed in Benda's modification of Flemming's fluid, and stained by the mitochondria method of Benda, or by the neutral gentian method. Under these circumstances the *B* cells are seen to be filled with minute violet stained granules. The *A* cells are best demonstrated by fixation in acetic osmic bichromate and staining in acid fuchsin followed by differentiation in solution of methyl green. In these preparations the *A* cells are seen filled with minute granules stained red, while the *B* granules remain unstained. After this fixation also the *A* granules may be stained in safranin or in gentian violet.

Both the vital stains and the section methods referred to above show that in the pancreas of the toad, particularly in that portion which is near the spleen, there are large numbers of single islet cells located in acini. These are mostly of the *A* type. None of these methods, however, shows cells containing both the zymogen granules and islet granules of either type, nor cells containing only a small proportion of islet granules as might be expected in cells undergoing transformation from one type to the other.

The pancreases of toads which have been kept for a long time under the influence of secretin, and in which the pancreas is wholly discharged, when examined after vital staining with neutral red, or with janus green, do not differ as regards the islet tissue from normal resting toads. The islets appear to be about as numerous and to present the same variations in size as in the resting pancreas. Nor do the sections made by methods which preserve the granules of the islet cells, and stained by methods which bring out these granules show in the pancreases of toads which have been under continual secretin stimulation for from four to seven days any increase in the number of islet cells in the acini nor any general transformation of acinus cells into islet cells.

The question then arises, what are the cells obtained in large numbers by Dale by this method of experimentation? I cannot answer this question positively because Dale does not describe the characters of his cells except to say that they are islet cells. But they cannot be islet cells because they do not contain islet granules. They differ from other acinus cells, as one can see from Dale's figure, in not staining in the basic dye. I have found in my preparations of the pancreases of secretin toads, acinus cells of two sorts which do not stain with basic dyes. Whether either of these corresponds to those obtained by Dale or not I am unable to state, because Dale does not give any description of his clear cells except to say that they are islet cells. One type of acinus cell which I have found sometimes present in large numbers in my preparations made by the method recommended by Dale, owes its failure to stain with toluidene blue to post mortem changes. These cells show, instead of the usual deeply stained basal zone, a pale stained protoplasm in which no zymogen is visible and which has a coarse alveolar structure. Examination of these cells by methods suitable for the demonstration of mitochondria shows that the granules of this type also have disappeared or are disappearing. The nucleus shows also a certain degree of chromatolysis. The change which these cells have undergone can be produced in any desired degree by simply keeping the pancreas in normal salt solution and it may be observed going on gradually under the microscope if fresh preparations mounted in salt solution are observed continuously for one-half to one hour. It does not affect all cells of the acinus at the same time. Sometimes a single cell will show the change, and at other times only a single cell retains the normal structure. Sometimes large areas of the section may show this change.

The other type of clear cell owes its lack of staining power to a true exhaustion of the chromophile substance of the pancreas. In these cells the nucleus shows no degenerative changes, the mitochondria are well preserved, but the cell differs from the normal resting cell in the absence of zymogen granules and in the great reduction of the basophile material of the base of the cell. These cells, however, are in no sense islet cells, because they do not contain the characteristic granules of the islet cells and they differ from the islet cells in their nuclear characters and in the nature of their mitochondrial apparatus.

As a result of my observations and experiments I am thus forced to conclude that prolonged activity provoked by the injection of a solution of dog secretin into the dorsal lymph sac of the toad is without influence on the number of islets, or on the quantity of islet tissue in the pancreas of this animal, as it is without influence under similar experimental conditions in the mammal.

3. *The effect of inanition on the number of islets of Langerhans in the pancreas of the guinea pig*

The claim that the islets of the pancreas are increased when exhausted by prolonged hunger was first advanced by Statkewitsch ('94) in connection with his general studies of the effect of starvation on various tissues. This claim was again advanced by Dale ('05), who based his conclusion on the examination of a single animal, a cat, concerning whose condition he judged on the basis of its extreme emaciation and the emptiness of its stomach and small intestines. In the pancreas of this animal he says that "sections from the splenic end showed a pancreas of the discharged type, though a few zymogen granules were present. There was a great abundance of large islets with clear evidence of progressive formation, as in the gland exhausted by secretin. . . . . The examination of this one specimen entirely corroborates the statement of Statkewitsch." The point of view from which this statement proceeds is well brought out by Starling in his "Recent advances in the physiology of digestion" where he says (p. 100):

> Complete exhaustion thus causes, not only an extrusion of the whole of the secretory granules, but also an emptying out and disappearance of the whole of the basophile protoplasm. It is worthy of note that the proportion of islet tissue to secreting tissue is increased, not only by the prolonged activity, but also by the prolonged inactivity which occurs during starvation. In the latter case the gland, which is not required for digestion, is called upon to give up its stored material, whether granules or protoplasm, to serve as food for the working of those parts of the body whose continuous activity is a condition of the maintenance of life. In the process of wasting the same changes are brought about in the appearance of the cells as when the discharge of their constituents is required for the production of a juice for the purpose of digestion.

Meantime the same statements have been advanced by Vincent and Thompson ('07) and by Laguesse ('10). Vincent and Thompson made the effect of inanition the subject of experiment in dogs, cats, pigeons and frogs. Of the result of these experiments it is impossible to judge, for the authors content themselves with general statements, and give no indication of the number of experiments made in each case, nor of the character of the numerical comparisons, except the statements that in the cases of two dogs the ratio of islets in a field of the resting pancreas to those in a field of the inanition pancreas is 3 to 16, and that in the pigeon by cutting out and weighing the pieces of paper in drawings of ten successive sections they found the ratio of islets in the inanition pancreas to that of the resting pancreas as 4.22–0.93. One cannot avoid, under these circumstances, asking how many normal resting pancreases were actually counted in each species in order to establish the normal range of variation and what were the maximum and minimum counts.

Laguesse ('10) on the other hand, has proceeded with his usual caution in similar experiments on pigeons giving the records in full of the counts made in single fields of the microscope of two series of pigeons which he had previously acclimated to the laboratory. Each series shows in sections taken at random from corresponding portions of the pancreas a higher average number in the inanition pancreases. It is noteworthy, however, that the controls of one series are as high in islet content as the inanition pancreases of the other series. Laguesse's results, while entitled to the highest consideration by reason of the care with which they were carried out, are still subject to the objections raised against the method of estimation of islet content by the examination of isolated sections, namely, that this method involves primary assumptions as to the equality of distribution of the islets in a given portion of the pancreas, which, in the guinea pig, I have proven by actual counts and weighings not to be well founded. Furthermore, there is always in this method the subjective element of unconscious selection, however scrupulous one may be in the selection at random of the sections to be counted.

In attempting to determine for the guinea pig whether inanition has had any effect on the number of islets in the pancreas I have employed the method of total counting of the islets after staining them by the neutral red injection method. The islets stain just as well in the inanition animals as in normal resting animals or as in secretin animals but the counting is not so easy, because of the fact that certain products of retrogressive change make their appearance in the cells, in the form of large globules which stain with neutral red. Always, also, there is considerable zymogen in the cells, the opacity of which helps to conceal the smallest islets, and in many cases numerous fat globules which have the same effect. The results, however, even if one makes allowance for a considerable loss of islets in counting, which is not the case, though doubtless the counts are somewhat lower than the actual numbers, are perfectly clearly in opposition to the claims that inanition increases the number of islets in the pancreas. Of the six animals counted, not one shows a high content of islets, thus

TABLE 5

Inanition experiments

Series 1. Inanition animals

INITIAL WEIGHT OF GUINEA PIG	NUMBER OF ISLETS	AVERAGE PER GRAM ANIMAL	DURATION OF INANITION
grams			*days*
535	27,654	51.69	5
537	16,721	31.13	6
840	19,704	21.07	7

Controls

487	25,583	52.7	
510	24,664	48.3	
845	28,540	33.7	

Series 2. Inanition animals

710	18,881	26.59	7
743	21,069	28.32	6
795	19,353	24.34	8

making it unnecessary to continue this objectionable form of experimentation. The time of withdrawal of food varied from five to seven days, water being supplied to the animals for the whole period.

There is clearly no indication in these cases of an increase in the number of islets as the result of the period of inanition. On the contrary, one might be tempted to conclude that the number had actually been reduced but for the fact that a reference to table 1 showing the islet contents in normal resting pancreases shows that large numbers of the normal animals have an islet content as low as, or even lower than that shown in these experiments.

4. Effect of inanition on the number of islets in the pancreas of the dog

In order to meet the possible criticism of the results in the guinea pig, that owing to its herbivorous habit the initial content of islets was large, similar experiments were made in dogs. Here again it was impossible to repeat exactly the experiment of Vincent and Thompson ('07) because they give no indication in their paper of the duration of the experiment, simply saying that "the difference between a section of a normal resting pancreas of a dog and one of a pancreas from a dog which has undergone inanition for several days is so striking as to render it almost incredible that observers could have disagreed on this point." Attempts to obtain convincing results by the examination of sections from corresponding portions of the pancreas of normal and inanition dogs having been unsuccessful in yielding constant differences, it seemed possible that the failure might be due to the comparison of animals which, owing to difference of age or of mode of life, were really not comparable. Accordingly, I made the effort to secure for this purpose litters of new born pups, rear them in the laboratory, and use them for this experiment. So far I have not had litters of sufficient numbers at my disposal to establish adequate controls among the members of the same litter, and have had in series 1 which follows to use for controls animals of the same age but of a different litter and much lower in weight. The neutral red technique did not prove so satisfactory in dogs as in the guinea pig, for the reasons that, although the islet granules stained intensely in the dye, the large amount of zymogen present and the greater size and solidity of the lobules of the gland rendered study by the teasing method more difficult. Moreover, in the dog I found it more difficult to secure a uniform staining of the whole pancreas than in other animals. Later a satisfactory method of counting for dogs was worked out, using Ringer's solution as the solvent and janus green as the stain. Teasing the pieces of the pancreas having proved unsatisfactory, the method of cutting thick sections of the pancreas with a Walb double-bladed knife having the blades separated 2 mm. was adopted. This method, I believe, permits a fairly accurate estimation of the islet content, if the pieces are collected and weighed after the counting and a ratio established between number of islets and unit weight. Unfortunately this method was developed too late in the work to permit of its application to more than one series of dogs of the same age. This series (no. 2), however, I believe, gives a fairly accurate idea of the frequency of islets in the pancreases in question. I do not feel the same confidence in the results offered in series 1, which were counted by the neutral red technique, as regards the actual content of islets, but as the several pancreases in this list were counted under the same conditions the relative error is probably about the same and the series may be accepted as showing the relative numbers of islets of Langerhans in the several pancreases, though probably much below the actual numbers per milligram in each case. In any case, there was no difficulty in seeing and counting the large islets and if Vincent and Thompson's claims that the islets were much increased in size as well as number were true, the counts should show this to be the case. As the period of Vincent and Thompson's experiments was not known, the duration of the first series of experiments was ten days, but in the second series, in view of Laguesse's statement that five days was the optimum period for producing the result, this period only was allowed to elapse from the time of withdrawal of food to the time of counting the islets.

In this connection it should be mentioned that animals kept without food will eat their own faeces or anything else which may be present in their cages. Accordingly all animals intended for this experiment were kept in special cages over a wire grating which permitted all refuse to drop through.

In table 6 each number represents the average number of islets per milligram in a series of sections 2 mm. thick taken at regular intervals in the corresponding portions of the pancreas. The splenic division includes the whole of the swollen extremity of the omental division of the pancreas, the body the narrow portion between the preceding division and the pylorus, the duodenal division all the pancreas included in the mesoduodenum.

TABLE 6

Showing number of islets of Langerhans in the pancreas of dogs after inanition, in terms of number of islets per milligram of pancreas

Series 1. Inanition dogs

INITIAL WEIGHT OF DOG	SPLENIC DIVISION	BODY	DUODENAL DIVISION	DURATION OF INANITION
grams				*days*
12,150	8.08	6.2	5.9	10
10,170	10.12	5.82	4.58	10
*Control**				
7,406	5.26	3.59	6.99	
6,200	9.41	7.12	5.86	
Series 2. Janus green method Three dogs of same litter, aged four months				
Inanition animals				
6,000	16.25	23.83	12.7	5
6,100	12.92	16.96	8.89	5
Control (twenty-four hours fast)				
5,300	16.8	32.87	15.8	

*In this series the two inanition dogs were members of the same litter, the two controls were members of another litter. All were reared in the laboratory and were approximately six months old.

Thus, in dogs as well as in guinea pigs my counts offer no support to the idea that after several days' withdrawal of food the number and size of islets in the pancreas are increased.

THE RELATION OF THE ISLETS OF LANGERHANS TO THE DUCTS AND ACINI OF THE PANCREAS

The majority of workers are agreed that the islets when fully formed have no connection with the ducts, although many admit that they are formed in part from the duct system. The connection with the duct system is, however, a logical necessity for those who maintain that islets and acini are interchangeable structures, while, on the other hand, the proof that they were separate from the ducts would be a strong argument in favor of their having a function distinct from that of the rest of the pancreas. It is not surprising, therefore, that this question has been the subject of considerable discussion. A full account of the observations which have been made and of the views which have been expressed concerning it, will be found in Laguesse's summary of the literature ('06–'08). It will suffice here to point out that there is evidence of a reaction against the view of independence not only in the observations recorded by those who believe that islets may be transformed into acini and acini into islets, but also in the form of actual demonstrations of continuity. Laguesse, perhaps, takes the most advanced stand on this question for he says that direct connections with the ducts are the rule rather than an occasional exception, and that many islets may have several such connections. Laguesse ('10) has also demonstrated in the clearest way the continuity of islet tissue and acinus tissue as well as the connection between intralobular ducts and islets, supporting his descriptions by drawings of series of sections which show unmistakably direct contact of islet cells and acinus cells without any intervening connective tissue septum.

Weichselbaum and Kyrle ('09), also, have observed islets in connection with the ducts in the human pancreas, particularly in young subjects and the general purport of Helly's work ('06) on the histogenesis of the pancreas is in the same direction. These authors, however, do not express clearly their views as to the frequency of this connection in the adult pancreas, though they record experimental observations which indicate that the duct epithelium possesses the power of forming islets even in adult life.

On the other hand there are many authors who not only deny any connection of the islets with the ducts but even maintain that those located in the lobules of the pancreas are wholly independent of the surrounding acinus tissue, being separated from it by a complete fibrous capsule.

The facts brought forward by Laguesse on the one hand, and by Weichselbaum and Kyrle on the other, relative to their observations of the occurrence of direct connections between the duct and islets, admit of no discussion, for the illustrations furnished by the authors are too clear and convincing to be evaded. The only question that may be discussed is the frequency of these occurrences. At first sight, taking into consideration the negative results of the injection and impregnation methods, it would seem probable that the cases observed by Laguesse and by Weichselbaum and Kyrle were simply instances where the islet had failed to complete its development and to separate from the mother tissue. But, when one considers the difficulty of demonstrating such connections with ducts, at least by the study of serial sections, the suspicion seems justified that many more connections escape notice in a series than are observed.

Laguesse ('06–'08) himself discusses the possibility of the connections breaking temporarily or even definitively in islets about to disappear, but after his latest studies is not willing to admit this, even, he says, though he remain alone in maintaining that there is a connection.

Laguesse and his pupils, as a matter of fact, do stand alone in claiming that the islets are usually connected with ducts or with acini, though others, who believe that, in general, they are separate, admit the occasional occurrence of such connections. Pearce ('03) describes the separation of islet and acini as occurring at about the third month in the human embryo, but finds solid cords of cells connecting them in a case of syphilitic pancreatitis of the new born. Continuity of acini and islets are described by

Dale ('05), Vincent and Thompson ('07), Mankowski ('01), Tschassonikow ('00), Dewitt ('06) and others. But with the exception of Laguesse, Weichselbaum and Kyrle, and Helly, I have found no authors who have seen direct connections with the ducts, though Lewaschew ('86) in his injections from the pancreatic duct succeeded in forcing the mass into islets.

By means of new methods for vital staining of islets and ducts I have been able to show that the relations of islet to duct in the guinea pig are precisely as described by Laguesse, and that while there is a certain number of detached islets, the rule is epithelial continuity between islet and duct or between islet and acini or both. This has been accomplished by means of the combined staining of the pancreas with pyronin and neutral red, or pyronin and janus green, or methylene blue and neutral red. The methods for using these stains are described under technique, and it is necessary here merely to state that as far as the epithelial elements of the guinea pig of the pancreas are concerned, pyronin or methylene blue intra vitam stain only the duct cells, leaving the others colorless. The stain is a diffuse one in the epithelial cells, and strangely enough the nuclei stain, though the nuclei of other cells in the pancreas remain unstained. The intensity of the stain is greatest in the smallest ducts and diminishes in intensity as the size of the duct increases. The centroacinous cells, as might be expected, stain intensely. By using two dyes together, a double stain may be obtained, enabling one to delimit exactly the two tissues, islet and duct. The pyronin preparations must be studied fresh, but as the dye reduces slowly in the tissue, the preparation may be kept for several hours. Treatment of the preparation with ammonium molybdate makes the dye somewhat more resistant to glycerine, but does not protect it from alcohol. Glycerine mounts last for several days but gradually diminish in value. The methylene blue preparations, on the contrary, may be made permanent in the usual way by ammonium molybdate, this method also permitting imbedding in paraffin, and sectioning. Total mounts are, however, most valuable because they enable one to dispense with reconstruction and to see the whole plan in a single specimen.

These methods have brought to light in the pancreas of the guinea pig a system of tubules which also have escaped the injection methods, and which appear to be of considerable importance by reason of their relation to the growth of pancreatic tissue. This is a system of fine tubules which, by forming anastomoses, connect together adjacent sections of the duct and neighboring branches of the duct.

This system was studied to some extent by Gianelli ('98) and by Laguesse, the former regarding them as constituting a sort of embryonic tissue endowed with the function of furnishing new pancreatic acini. By the majority of observers they have been confused with the system of glands attached to the ducts.

As shown in my preparations made by means of pyronin or methylene blue, this system is composed of a series of tubules of small calibre, which take their origin from the duct or from the larger branches of the duct, and which branch freely in the connective tissue surrounding these ducts. The branches anastomose freely with one another (fig. 4), and so form, along the main duct and its primary branches, a web of extreme intricacy, binding together the successive sections of the duct, and also connecting primary and secondary branches with one another, even at some distance from the main duct. From this branching system come off many short branches which end blindly.

The tubules of which this system is composed vary from 12–27 micra in diameter, though they may be thicker than this at certain points where small islets are attached, or where a mucous gland takes its origin from the tubule. In some cases the tubules take the form of a highly branched tubular gland (fig. 5). The usual arrangement is well shown in fig. 4, although the course of the tubules is in reality much more tortuous than in the figure, which is made from a preparation in which the tissues are stretched to make the preparation thinner and more transparent. The small projections seen at intervals along the tubules are chiefly mucous glands, though some are small islets of Langerhans

The number of these tubules varies in different guinea pigs, and at different parts of the gland. In some guinea pigs the whole duct may be surrounded by a web of such tubules, some originat-

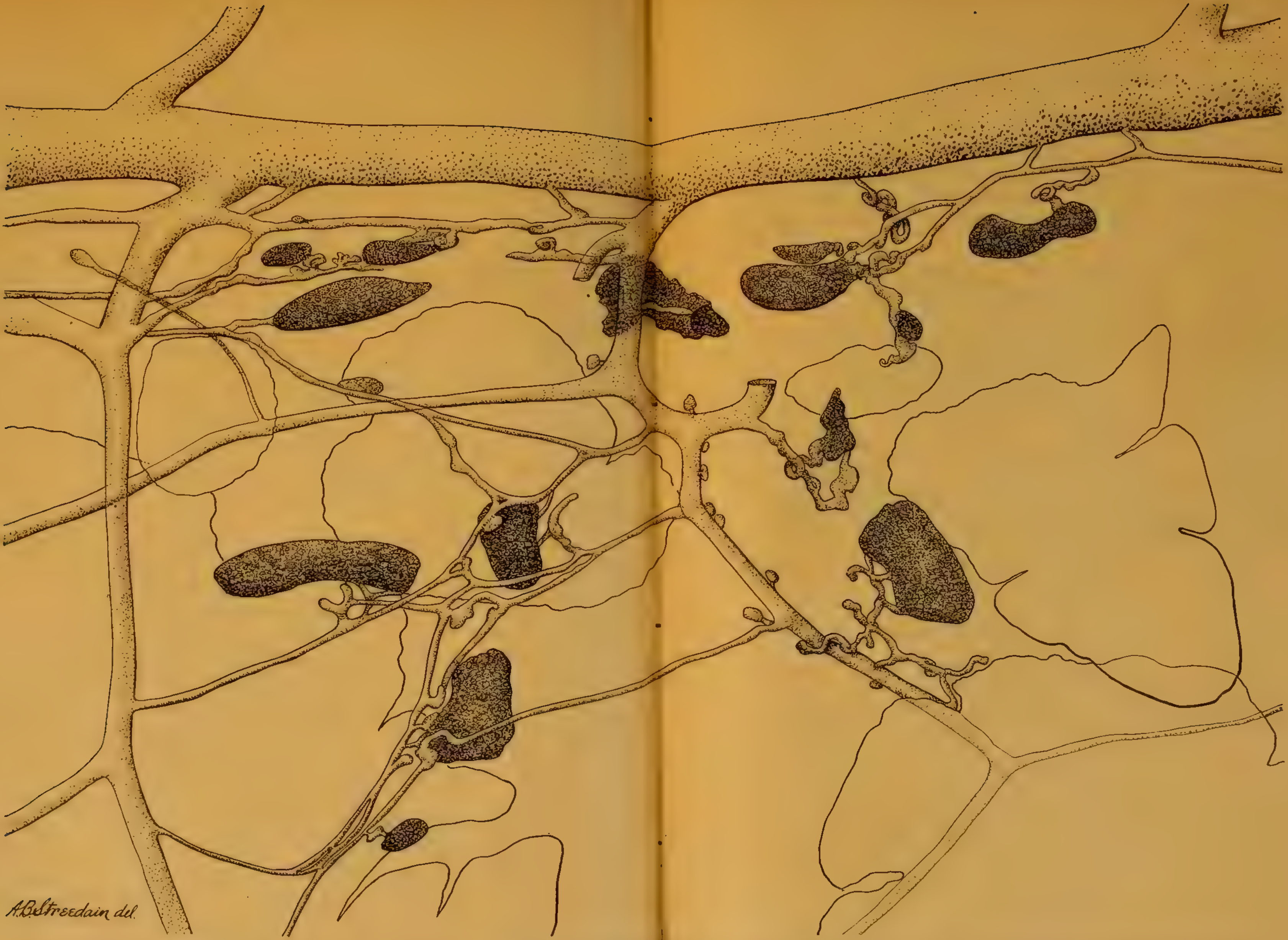


Fig. 4 Drawing of a preparation of the ducts of the pancreas of the guinea pig, stained by methylene blue injection, showing the system of anastomosing tubules around the duct and their relation to the islets of Langerhans. × 65.

ing from the main duct, some from its side branches In others there may be large sections of the duct which show no such branches, while at other points they may be abundant. Age seems to have little influence on the numbers.

The epithelium of these tubules is composed of low epithelial cells, which appear cubical when cut across, but are seen to be irregularly polygonal when viewed from the surface of the tubule, one axis of the cell being elongated in the direction of the long axis of the tubule. The nuclei, likewise, are oval, their long axis corresponding to that of the cell. A few mitoses may be observed in these cells. The protoplasm contains numerous mitochondrial filaments, which may be demonstrated by the acetic osmic bichromate method, followed by staining by the acid fuchsin-methyl green method. Except for these, the protoplasm appears homo-

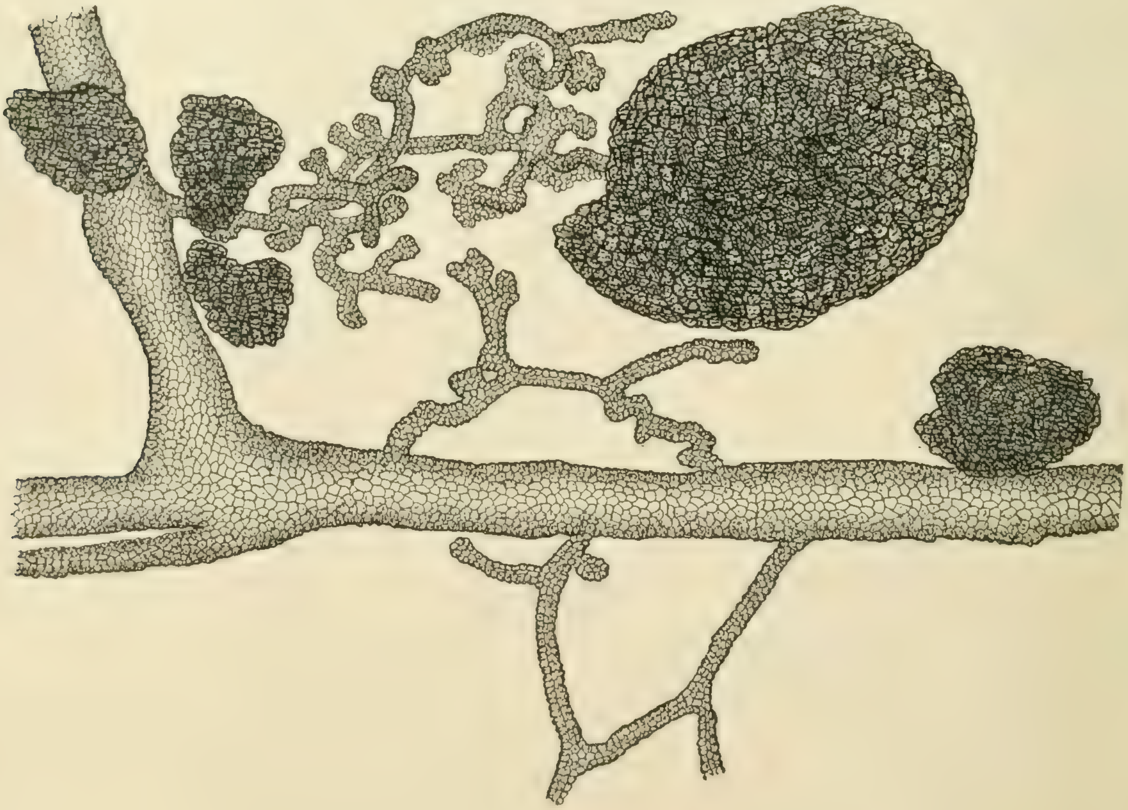


Fig. 5 Duct with branches showing the highly branched tubules connected with the duct and with an islet. Intra vitam staining with pyronin and neutral red. × 77.

geneous, unless it contains a secretion antecedent, in which case the portion of the cell along the lumen contains irregular granules which stain with mucicarmine. A few goblet cells may be present among the epithelial cells, though rarely are these found. At irregular intervals small flask-shaped mucous glands open into the tubules. The lumen of the tubules is continuous throughout, though, in places, it may be as narrow as 1.5 micra.

When stained in neutral red as well as pyronin, and studied under the oil immersion, some of the projections on the tubules are seen to be composed of cells which stain like islet cells, although some of these cells contain fewer granules than the typical islet cell. Single islet cells may also be seen here and there among the epithelial cells of the tubules. In addition to these islet elements, islets of every conceivable size, from the smallest to the largest in the pancreas, are attached to these tubules, either directly, in which case the islet is a rounded projection on the side of the tubule, or indirectly, when the islet is connected with the tubule by a longer or shorter stalk which is of the same nature as the tubules from which it springs.

Also attached to these tubules, but less frequently than the islets, are small pancreatic lobules, varying in size from a single acinus to a group of acini. In some cases these acini arise from the ducts which lead to the islets. In these acini, and in the small islets which are attached to the tubules, as well as in the tubules themselves, occasional mitoses may be observed, though these are rare except under those conditions where there is defect of pancreatic tissue as the result of removal of a portion of the pancreas, or in young animals in which the pancreas is rapidly growing.

The single islet cells which are to be found among the epithelium of these tubules may be interpreted as cells which have been differentiated out of the epithelium of the tubules, and similarly the regular gradations in size of the islets of this system, from the smallest to the largest, as well as the presence of small lobules of pancreatic acini attached to the tubules, together with the occurrence of mitoses in all the anatomical elements of this group, indicate that we have to do here with a tissue of a low order of differentiation, which is capable under proper conditions, of producing by differentiation, and by mitotic division, islets, acini, and mucous glands.

This function of producing islets, etc., is also shared by the columnar cells of the ducts of the pancreas, for it is by division of these that the tubules in question are being produced, and furthermore, islets, and acini in process of growth are related to these ducts in precisely the same way as they are to the anastomosing tubules which have just been described. The ducts of the gland-like outgrowths which are found all along the pancreatic duct are also composed in part of undifferentiated cells like those of the duct and one may find islets or acini originating from these (fig. 6).

As shown by the vital staining methods the islets of Langerhans of the guinea pig pancreas fall into the following categories:

1. Islets unconnected with the acinus tissue, located in the interstitial tissue of the pancreas, particularly along the duct and its primary branches, and connected with the ducts either directly by short ducts, or indirectly by means of the system of tubules which has just been described.

2. Islets located in the substance of the pancreatic lobules, but wholly unconnected with the acini, and directly connected with the interlobular duct system by shorter or longer branches.

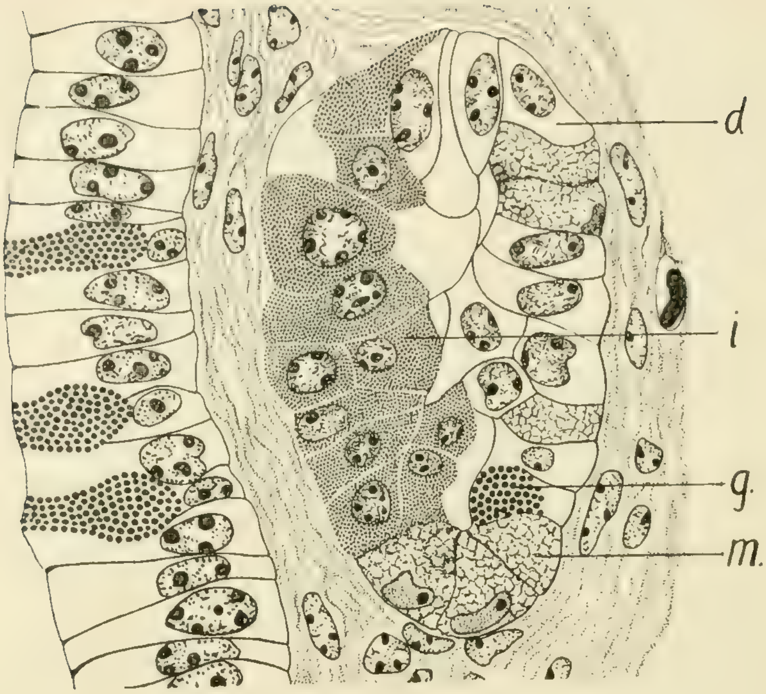
3. Islets located in the lobules of the pancreas, with the acini or ducts or both of which they are in direct continuity.

4. Islets unconnected with either acini or ducts. These may be located either in the interstitial tissue or in the substance of the lobules. Islets of this class belong primarily to 1 and 2, since having originated from a duct they have lost this connection.

The islets of class 1 are of all sizes, varying from single cells to the largest islets of the pancreas. The mode of their attachment to the ducts is well shown in figs. 4, 5, 7, and 8. As shown in fig. 7 many of these islets have multiple connections with ducts, indicating that they have either originated by the fusion of several islets of separate origin, or effected secondary unions with the duct system. In rare cases only does the lumen of the duct actually penetrate the substance of the islet, and in the few cases where this does occur, as a rule, the proximal portion of the islet into which the lumen extends is composed of duct cells, not of islet cells. The islet is thus, notwithstanding its direct connection with the duct, unquestionably a ductless gland, and the duct connections are significant only as an indication of the source whence the islet has been derived. The few cases in which an islet is penetrated by the lumen of its duct are of interest as explaining those cases described by Weichselbaum and Kyrle ('09), where cystic islets have been found as a result of occlusion of the duct. Even the smallest islets on the duct system are solid, that is, the duct does not enter their substance (fig. 9).

The islets of class 2, namely, those which, though imbedded in the substance of the lobules, yet have no connection with their substance, are apparently islets of the same sort as those which constitute class 1, but which have been surrounded secondarily by acinus tissue. These islets are of interest because, having been secondarily enveloped by the acinus tissue, they are surrounded by a thicker and more continuous connective tissue layer than those which have originated within the lobule, and so are responsible for the ideas of those who think that the islets of Langerhans are everywhere separated from the surrounding acinus tissue by a fibrous capsule.

Class 3 contains the great majority of all the islets of the pancreas. These islets are located directly in the substance of the pancreatic lobules and are continuous with the ducts and acini of these lobules usually at many points of their surfaces. I have studied the relation of these islets to the other epithelial elements of the lobule both in the freshly stained preparations from pyronin and methylene blue injections, and in sections of methylene blue preparations after fixing in ammonium molybdate, etc., and have confirmed the results by the study of serial sections of material fixed in the acetic osmic bichromate mixture and stained in fuchsin-methyl green, which permits an accurate diagnosis of all the epithelial elements of the pancreas. As a result of these studies I have come to the conclusion that the relations of the islets of this class in the guinea pig are substantially the same as those described for man by Laguesse ('10), with the important exception which I shall discuss more fully later, that I find no transitions between islet cells and acinus cells, and no acini which are



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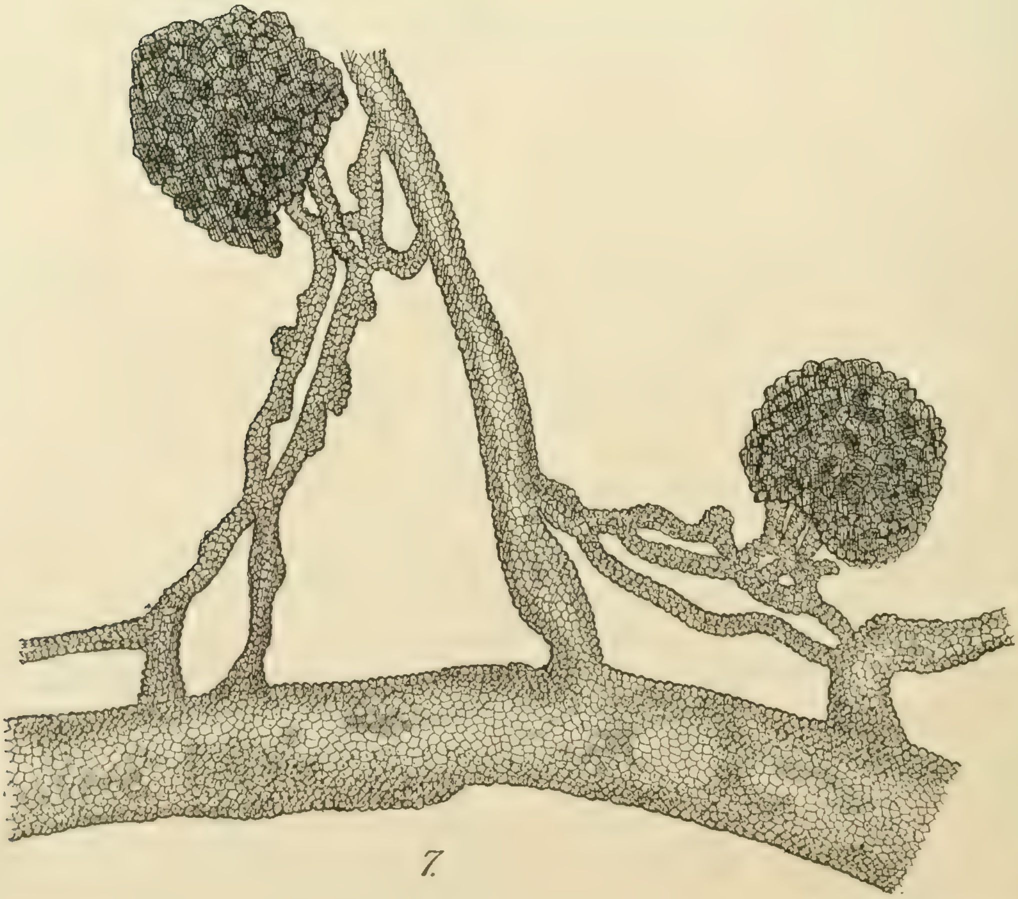

7.

Fig. 6 Section of gland near its origin from the duct, showing several types of epithelial cells. Acetic osmic bichromate, safranin, acid violet; *i*, islet cells; *m*, mucous cells; *g*, goblet cell; *d*, undifferentiated epithelium.

Fig. 7 Duct from pancreas of the guinea pig showing multiple connections with islets. Pyronin, neutral red. × 77.

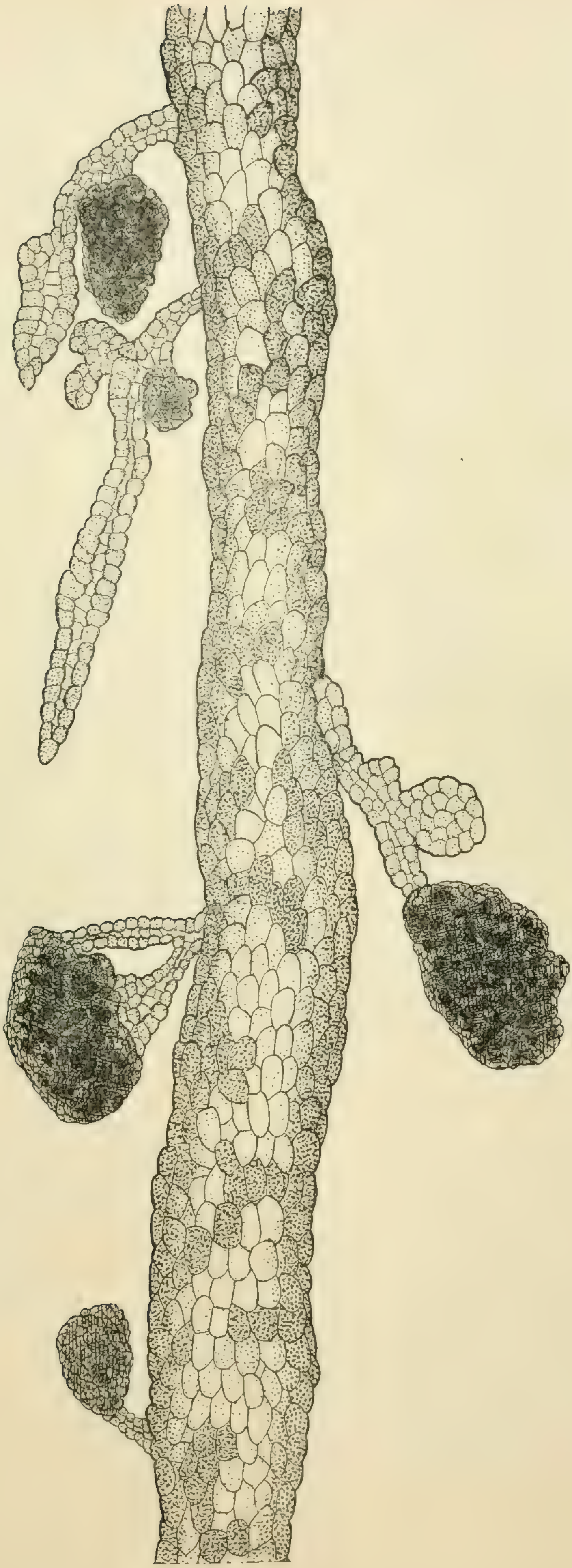

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Fig. 8 Duct of pancreas of guinea pig showing origin of islets from short branches and from glands. × 256.

separated from the duct system. The examination of preparations fixed in Zenker's fluid and stained by Mallory's aniline blue method for connective tissue, shows that the apparent continuity between islets and acini or ducts is real, and that no connective tissue fibrils are interposed between the islet and the acini with which it appears to be continuous. In the islets of this class it is impossible to define a capsule, though such a structure may with justice be ascribed to the islets of class 2.

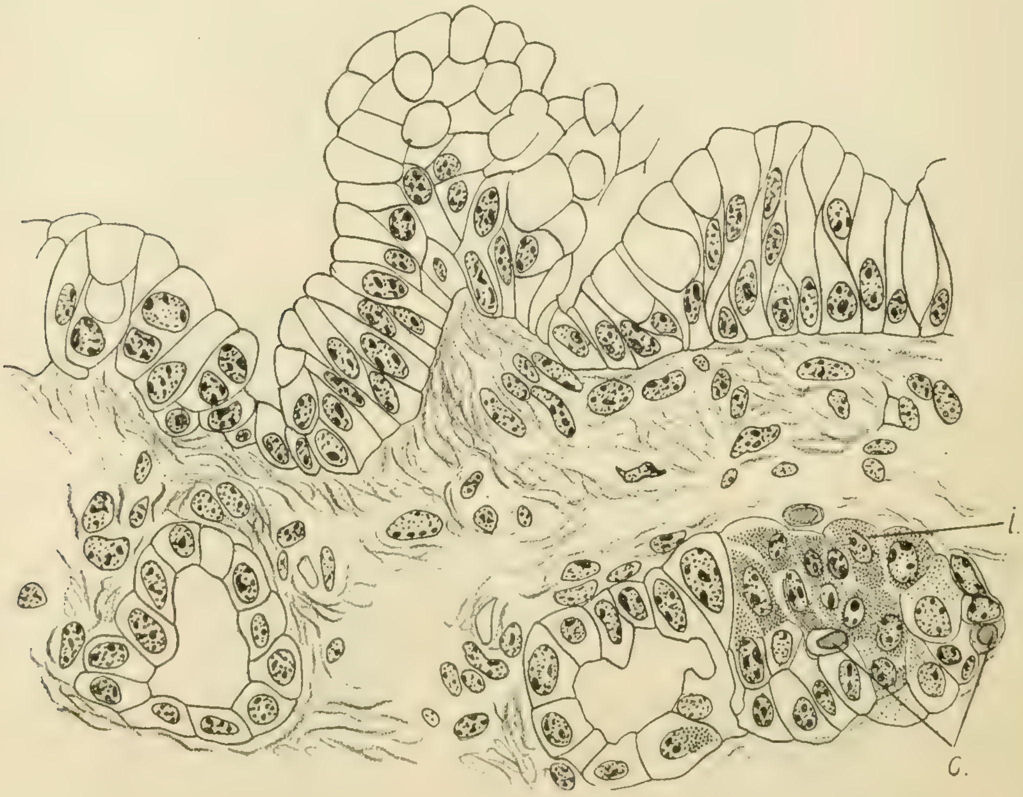

Fig. 9 Section through a small tubule near its origin from the pancreatic duct, showing origin of islet cells from undifferentiated epithelium. In some of the islet cells only a few granules are seen. Guinea pig; chrome sublimate, neutral gentian; *c*, capillaries; *i*, islet cells. × 533.

The islets of class four are those which have lost their connections with ducts. It is impossible to judge from my preparations how frequently this separation occurs, for the pressure necessary to flatten the preparation so as to spread the tissues apart may have broken the connections. This has happened frequently, for one can still see the torn duct going in the direction of the islet. In other cases no trace of a duct can be observed. These are, nevertheless, few in number and exceptional. Fig. 4, for example, shows fourteen islets each one of which is directly connected to one of the branches of the system of tubules surrounding the duct.

In his recent article Laguesse ('10) in discussing Dewitt's description ('06) of islets in the interstitial tissue, expresses doubt of their having been composed exclusively of islet tissue and suggests that, if continuous series of sections of these islets had been studied, they would have been found to be partly composed of acini. This is, of course, of some importance to Laguesse's theory of balance, for the occurrence of islets with no connection with the acinus tissue would be difficult to reconcile with the view that islets and acini are subject to a constant reciprocal transformation. In the preparations stained intra vitam with neutral red it is easy to see that Dewitt's description is correct, and that these islets are composed solely of islet cells. In order, however, to be perfectly sure on this point I have studied these islets in complete series, and found them wholly free from acinus tissue. This is, moreover, what might be expected in view of their origin, for it is apparent that they originate directly from the duct and at no period of their history have any direct relation to acini.

It will be seen that the observations just described furnish an explanation of the conflicting views of different authors concerning the true anatomical relation of the islets of Langerhans to the ducts and to the acinus tissue, for all of the various kinds of relation which have been described actually occur. That the connections with the ducts have been so uniformly overlooked is doubtless due to the fact that serial sections have not been studied with sufficient care, and because, even in serial sections, the connections are hard to find because they are so small. The failure of the injection method is doubtless due to the fact that the lumina of the ducts going to the islets are filled with a viscid mucus which prevents the injection mass from penetrating to the islet, and that there is no terminal cavity into which this secretion can be backed up. The pyronin method, however, furnishes a means by which any one who may still have doubts about the connections of islets with ducts in the guinea pig may dispel these doubts by an experiment which takes less than twenty minutes to perform.

The source of the idea that islets of Langerhans are separate from acinus tissue, and surrounded by a special capsule is to be found in the islets of class 2, which have both of these characters because, though contained within the lobule, they are not really part of it, but have intruded into it from outside. These islets are among the most conspicuous of intralobular islets, and because of their sharp separation from the surrounding acini, and also of their size, are most likely to be regarded as 'typical' islets and so selected for study by those investigators, who have not at their disposal a technique which permits of the accurate diagnosis of islet cells. It is true nevertheless that the majority of these islets are connected with ducts, though the same obstacles to the discovery of these connections are present as in the case of the interstitial islets.

THE STRUCTURE OF THE PANCREATIC EPITHELIUM

Having considered from the standpoint of quantitative study the claims that have been advanced with respect to the possibility of transforming acinus tissue into islet tissue by experiment, with results which are wholly opposed to these claims, it remains to consider the other evidence of transformation which has been brought forward, namely the statement that, between the cells of the acini on the one hand and those of the islet on the other, are to be found cells of intermediate characters, which may be regarded as transitional elements. If such transitions occur, then the quantitative results merely indicate that a balance is maintained between the construction and deconstruction of islets. It is necessary, therefore, to consider in detail the descriptions of transitions which have been given, and to inquire to what extent each satisfies the logical requirements of the case. Before proceeding to this discussion, however, it is necessary to know the nature of the cells between which we are seeking transitions. It has been pointed out in the introduction to this paper that, with few exceptions, workers in this field have accepted a definition of islet cells by negative characters. We are now able to substitute for this a positive definition and to consider in the light of such a definition the transitions which have been described. I shall describe first the zymogenic cell, then contrast with this the two types of islet cells, and those of the smaller ducts, and centroacinous cells.

1. *The cells of the pancreatic acini*

In the structure of these cells the following elements require consideration: zymogen granules; prozymogen granules; the basophile substance; mitochondria; internal reticular apparatus; the continuous substance of the protoplasm; and the nucleus.

When a preparation of the fresh pancreas of the guinea pig mounted in guinea pig serum is examined under a high power the characteristic division of the pancreatic cell into two zones is seen. The central zone of the cell, varying in width with the stage of functional activity, is closely packed with coarse granules which are usually known as zymogen granules. The basal zone, under the low power, or under high power dry lenses, appears perfectly transparent and homogeneous, though small fat droplets may be present. Under a good apochromatic oil immersion objective, this basal zone exhibits an indistinct striation, parallel in direction to the long axis of the cell and perpendicular to the basement membrane. This striation was first observed by R. Heidenhain, and is due, as will appear later, to the presence of long mitochondrial filaments or rods (chondrioconts) similar to those demonstrated in the tissues of embryos by Meves ('08). The substance between these filaments appears homogeneous in the fresh cell even under the best apochromatic objectives, and exclusive of the mitochondrial filaments mentioned above exhibits no structural elements whatever. It should be pointed out here that the mitochondrial filaments have no relation to the so-called basal filaments of Solger which belong to the homogeneous basal substance. Under certain conditions, there may be seen in the fresh cell, better in cells stained with neutral red, at the junction of the basal zone and the zone of zymogen granules an intermediate zone of small granules of irregular shape, which I regard as zymogen granules in process of formation, and therefore call prozymogen granules. These must not be confused with the basophile substance of the cell which has elsewhere been called 'prozymogen' by Macallum and myself.

When stained with neutral red and examined fresh in salt solution the basal zone remains quite unstained. The zymogen granules stain faintly red in dilute solutions, but may be made to stain strongly by using stronger solutions of the dye. Resting acini show a clear basal zone which is unstained, and a distal zone containing coarse zymogen granules which are faintly stained. In the active gland, as for example, after a few hours' secretin stimulation, or after a meal, a new element makes its appearance, between the zymogen granules, which are now reduced in number and size, and the basal clear zone. These are the prozymogen granules referred to in the preceding paragraph. They are particularly conspicuous in the preparations stained with neutral red because they stain much more intensely than the zymogen granules. After prolonged secretion, the zymogen granules have wholly disappeared and the secretory content of the cells is reduced to a narrow row of these prozymogen granules along the lumen. If the stimulation is continued long enough even these disappear. I regard these granules as young zymogen granules for the reason that they wholly disappear from the pancreatic cells after a sufficient period of rest, while a few hours' stimulation will cause their reappearance in the usual situation.

In preparations stained in dilute solutions of janus green, as was first pointed out by Michaelis ('00), there appear in the basal zone, distinctly stained, the structures which were responsible for the faint striation observed by Heidenhain. These structures are, as Michaelis recognized, identical with the elementary filaments described by Altmann ('94) in the pancreatic cell, but as Michaelis also correctly recognized, have nothing to do with the structures which have been variously termed basal filaments, ergastoplasma, etc. In the janus green preparations these elements may be seen as independent rods or filaments resembling bacilli, located for the most part in the basal portion of the cell.

In studying the several components of these cells by fixing and staining methods it is necessary to consider those elements which are visible in the living cell or in the vital stained preparations, and to discard methods of fixation which destroy one or more of these elements, or at the most to use such methods for experimental purposes only. On the other hand, methods which introduce new structures into the cells which are not visible in the living cell must also be discarded. Up to the present, these conditions have usually not been met, as is sufficiently indicated by the almost universal confusion which exists with reference to the identification of the striations observed by R. Heidenhain in the living cell. Many observers identify these with the so-called basal filaments of Solger which are not visible in the living cell, while in reality they are due to the filaments observed by Altmann and Michaelis. The reason for this confusion is that acid fixing fluids are usually employed, which destroy the filaments of Michaelis and Altmann, while they bring to light new filaments which were not visible in the living cell.

The fluids which I have found to preserve what is present in the living cell without introducing new structural elements by precipitation are as follows:

1. Benda's acetic-osmic-chromic fluid for the preservation of mitochondria. This, though a poor penetrant, preserves all the elements in the peripheral portions of pieces of pancreas fixed in it, and at the same time permits one to see the effect on the deeper portions of the tissue of the acetic acid which penetrates more rapidly than the other components of the mixture.

2. The acetic-osmic-bichromate mixture (Technique, 4 d). This mixture has all of the advangates of Benda's fluid, and the additional one that, by appropriate staining, it enables one to differentiate all of the epithelial elements *inter se* in a single preparation.

3. Lane's chrome-sublimate mixture.

4. Formalin Zenker (Technique, 4 c). This is the best fixing solution from the standpoint of penetration, but does not lend itself so well to differential staining as the preceding solutions.

In formalin Zenker the canals of Holmgren's system show best, though they may also be observed well in preparations fixed in Orth's or Kopsch's formalin bichromate solutions.

To make clear the structure of the acinus cell, I have made drawings to illustrate these components separately, as well as together. Fig. 10 shows a preparation fixed in chrome sublimate and stained in toluidin blue alone. In this preparation it will be seen that the inner zone of the cell is occupied by a very regular network, the spaces of which are those which contain the zymogen granules. This is, however, not a true network, but simply the optical section of the continuous cytoplasmic partitions which separate the granule-holding spaces from one another. The basal substance of the cell stains strongly in toluidin blue, and exhibits an indistinct longitudinal striation. The reason for this stria-

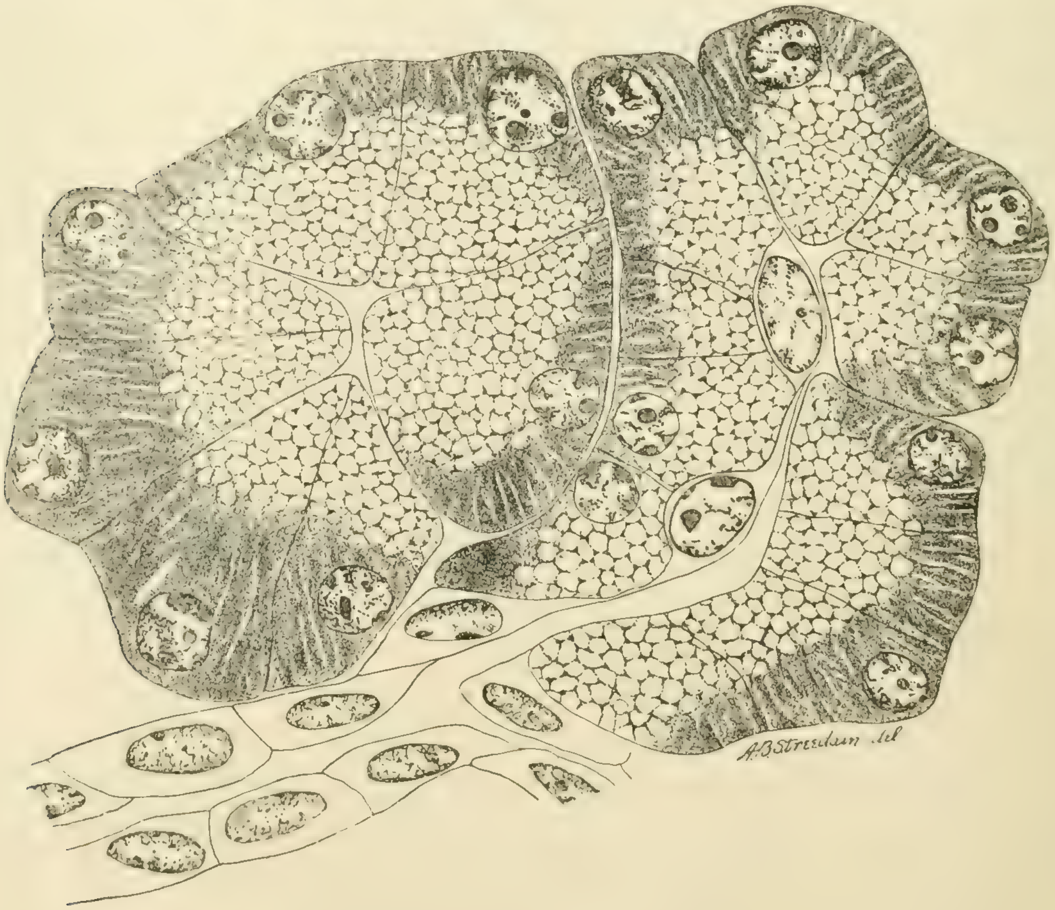

Fig. 10 Pancreatic acinus of guinea pig showing the homogeneous basophile substance, in which the light spots represent unstained mitochondria. In the central portions the zymogen-holding spaces. × 1555.

tion is apparent when one examines closely this portion of the cell under the high power, when it is seen that the striation is due to the fact that there are in the cell unstained areas shaped like the filaments observed in the fresh cell after staining with janus green. The striation seen in this preparation, then, is due to the fact that we have in the base of the cell a continuous basophile substance, in which rod-like elements are imbedded. When the latter are dissolved, they leave irregular spaces in the continuous substance. When they are preserved but remain unstained, the spaces they occupy appear as clear rod-shaped spaces in the continuous substances and so give a deceptive appearance of longitudinal striation.

In preparations which have been fixed in solutions containing a sufficient amount of acetic acid to destroy the rods, a totally different appearance is presented by the basal zone. In acetic sublimate or Zenker's fluid preparations, the whole of the basal basophile material is found to be broken up into a feltwork of fine filaments which are wholly different from the coarse rods seen in the janus green preparations. These filaments, while often running parallel to the axis of the cell, are also often found forming an intricate skein running transversely in the base of the cell. These are the familiar basal filaments of Solger or the ergastoplasmic filaments of Prenant, Garnier, Bouin, etc.

The question now arises whether the homogeneous basophile material seen in the chrome sublimate preparations or the fine filamentous material seen in the acetic sublimate fixations is the true structure present in the living cell, or to express it differently, whether the filaments seen in the acid fixations are pre-existent in the living cell, or artefacts produced by precipitation. I have tried to obtain an answer to this question, but without success. The most that I can say is that I have been unable to see these basal filaments of Solger in the living cell, though I have studied such cells in native serum and in salt solutions of different composition and concentration. They are not visible in preparations fixed in neutral formaldehyde, or osmic acid, or in fluids containing these substances provided the acetic acid is kept low. On the contrary they are visible in solutions containing a large proportion of acetic acid, as for example, Hermann's and Flemming's fluids 'and the solutions mentioned above. In preparations fixed in Benda's fluid the acini at the margin show a homogeneous basal substance while those farther and farther from the margin show the basal filaments more and more clearly. On the contrary, the filaments of Michaelis and Altmann become less and less apparent as one studies acini farther and farther from the margin in Benda preparations, which is a sufficient indication of a profound modification of the cytoplasm associated with the appearance in it of the basal filaments of Solger. I am therefore inclined to the opinion that the basophile filaments of Solger are fixation artefacts produced by acid precipitation, and that the real basal substance of these cells is homogeneous. It is quite possible, however, that the basal filaments are preëxistent in the living cell though invisible because they are imbedded in a substance of the same refractive index, and that they are rendered visible in the acid fixations by contraction. On this basis it would be necessary to assume that the filaments are swollen in the chrome sublimate, and formalin Zenker preparations, so as to occupy apparently all the space in the cell not taken up by filaments of Altmann, or fat globules.

Fig. 11 shows an acinus from a preparation fixed in acetic osmic bichromate, and stained in Altmann's anilin acid fuchsin, followed by differentiation in methyl green. In this preparation the filaments of Michaelis and Altmann show as distinct bacillus-like filaments, located for the most part in the basal portion of the cell and surrounded by a homogeneous basal substance. In these preparations the filaments are stained intensely red, the basophile substance green. The zymogen granules are also stained intensely red. This preparation corresponds exactly with what is seen in the living cell, and is the positive image where fig. 10 represents the negative.

Here, in particular, the filaments of Michaelis and Altmann require comment. These filaments were first observed in fixed material by Altmann ('94), who called them elementary filaments or vegetative filaments and who supposed that they were endowed with the power of growth, and of forming zymogen granules.

This observation of Altmann's shared in the general undeserved discredit which befell all of his work as a result of the attacks made on his methods by Fischer and others, and as a result of the prejudice aroused by the bold conclusions which he drew concerning the general nature of protoplasm. For these reasons Altmann's observations have for the most part been passed over in silence, or erroneously interpreted where they have been noticed at all. The new work on mitochondria is, however, daily making it more and more apparent that Altmann's works are full of valuable objective descriptions which can be verified perfectly in the living cell. Among those who have seen the filaments in question I find Laguesse ('06–'08), Michaelis ('00), and Babkin, Rubaschkin and Ssawitsch ('09). The fuchsinophile granules described by Théohari in the cells of the gastric glands, and by Launoy in the pancreatic cell probably represent imperfectly preserved Altmann filaments.

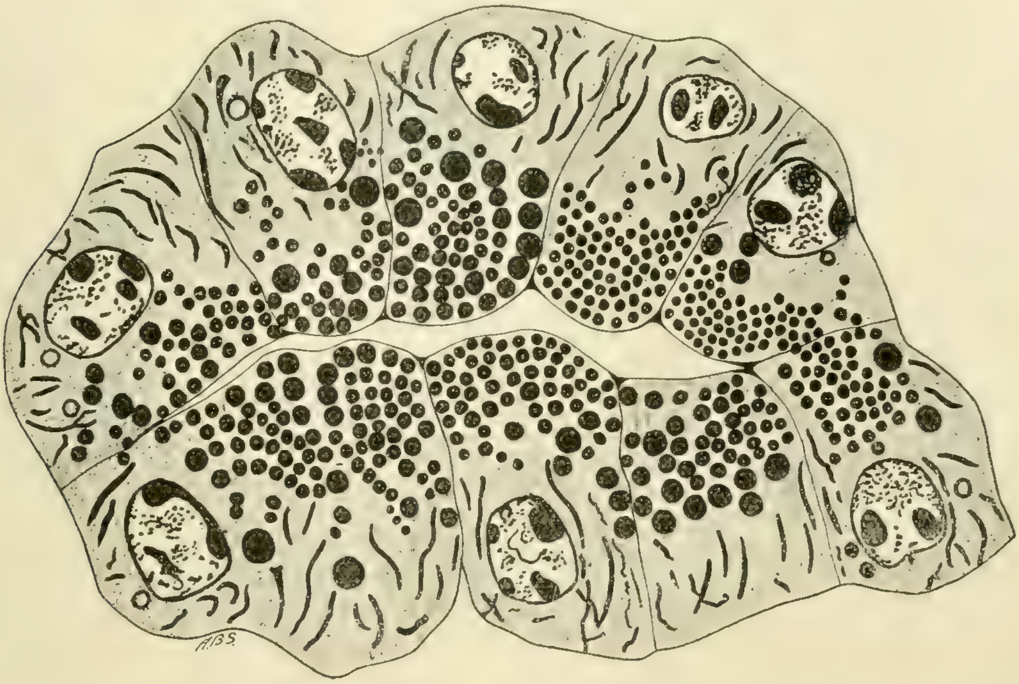

Fig. 11 Section of a pancreatic acinus of the guinea pig showing mitochondrial filaments imbedded in homogeneous basal substance. Acetic osmic bichromate, acid fuchsin, methyl green. × 1555.

Michaelis discovered these filaments in the pancreatic cell and the cells of other glands by means of vital staining with janus green, and correctly identified them with the filaments of Altmann. He also remarked that they were distinct from the basal filaments of Solger. Laguesse, on the other hand, while he observed them both by methods of his own and by Michaelis' method considered that they were related to the so-called ergastoplasma (that is, basal basophile substance or filaments), and called them ergastidions. Babkin, Rubaschkin and Ssawitsch ('09) gave excellent figures showing these structures but failed to interpret them.

Without doubt these filaments are responsible for the striations seen in the living cell by R. Heidenhain and others, for, using a 3 mm. apochromatic lens of 1.40 numerical aperture, one can see them perfectly in the living cell and recognize the exact parallel in structural characters between them and the filaments demonstrated by Altmann. Moreover, if janus green is added to such a preparation, while it is under observation, the structures may be seen to take up the dye until they stand out as sharply stained as they appear in an Altmann preparation. Thus, there can be no doubt of the vital preëxistence of Altmann's filaments.

The distinction between these filaments and the basal filaments of Solger or ergastoplasma is sufficiently obvious from their structure, the filaments of Altmann being coarse bacillus-like structures, the basal filaments on the contrary fine filaments which are much longer and which form an intricate skein. The basal filaments are always basophile, while the filaments of Altmann only stain in basic dyes when specially mordanted with this end in view. When fixed in non-mordanting solutions like neutral formaline they are distinctly oxyphile. Furthermore, it is possible to demonstrate the two kinds of materials side by side with differential staining, as was the case in the preparation from which fig. 11 is taken. Here the basophile material stains with the basic stain methyl green while the filaments of Altmann stain with the acid stain fuchsin. Similar results will be attained by other combinations of acid and basic dyes. For example, in a neutral combination of acid violet and safranin the basophile substance stains red in the safranin and the filaments stain violet in the acid dye. A mixture of pyronin and methyl blue gives a corresponding result. Furthermore, the independence of the filaments of

Altmann and the basal basophile filaments is proven by the fact that the filaments of Altmann occur in all the epithelial elements of the pancreas while the basophile filaments only occur in the acinus cells.

It is now apparent that these filaments of Altmann and Michaelis belong to the same category of cell-organs as Benda's mitochondria. Benda has admitted that a large number of the granules described by Altmann belong to this class, and Meves ('10) and Samssonow ('10) have shown by comparing the results of the Altmann technique with those of their own and Benda's technique on similar material that the several methods bring to light the same elements.

To this rule that the granules of Altmann, exclusive, of course, of pigment granules, fat granules, and secretion granules, belong to the mitochondria, the filaments of Altmann and Michaelis in the pancreatic cell are no exception. Like the mitochondria of the sex cells they may be demonstrated by the method of Benda. They are destroyed by acetic acid. They may be demonstrated by the methods of Meves, Altmann, and Regaud. Finally, I have demonstrated that in the spermatocytes janus green stains intra vitam only the mitochondria. Similarly in the pancreatic cell it stains only the filaments of Altmann.

The interesting question of the part played by these filaments in the secretory activity of the cell I shall discuss in a later paper. Here, it may be interesting to mention the fact that the small fat globules of the base of the pancreatic cell are imbedded in the substance of the filaments, which recalls the old observation of R. Heidenhain on the relation of fat to the basal filaments of the cells of the convoluted tubules of the kidney, and those of Altmann and his pupils on the formation and absorption of fat.

The canals of Holmgren may be studied in preparations fixed in formaline Zenker and stained with a basic stain. For this purpose a pancreas which has been exhausted by long secretion is best, for, in the resting pancreas, the territory of the cell which is occupied by the canals is so filled with zymogen granules that the canals are concealed. In the discharged cell, however, this place is occupied by chromophile substance and the canals may be seen as a network of closed spaces apparently excavated in this basophile material. The apparatus is located in the portion of the cell between the nucleus and the lumen but branches of the canals may extend basalwards along the sides of the nucleus. I have not been able to find any trace of a communication of these canals with pericellular spaces, either in these preparations or in sections prepared by the methods recommended by Holmgren and Kopsch and Golgi for the positive demonstration of the canals. Nor have I seen any continuation into them of processes of cells of the basement membrane or of centroacinous cells. I am therefore of the opinion that they constitute a system of closed spaces. I have elsewhere ('10) brought together observations in support of the theory that the canals of this system are the homologue in the animal cell of the vacuole which is so important an organ of the plant cell.

Apart from the filaments of Altmann (mitochondria) and the basal filaments I have not been able to demonstrate any structural elements of a fibrous nature, or any alveolar structure in the protoplasm of the acinus cell.

The nucleus of the acinus cell is spherical, and contains in addition to several large chromatin masses a well defined large oxyphile nucleolus.

2. The structure of the islet-cells

The islets of Langerhans of the guinea pig as shown by Diamare ('99), Schulze ('00), Dewitt ('06), and Lane ('07), consist of at least two types of cells, which Lane calls the *A* cells and *B* cells respectively. Many of the small islets are composed of *B* cells, which also are the most abundant cells in all the islets.

In the guinea pig, on account of the great size of some of the islets and on account of the fact that many of them occur in the interstitial tissue, it is easy to pick out an islet, detach it from the surrounding tissue and examine its cells in isotonic salt solution or in native serum. Under these circumstances the cells of the islet are seen to be crowded with extremely fine granules, which have a low refractive index, and so are difficult to see except with the best apochromatic objectives. In preparations stained intra vitam with neutral red or with janus green the cells of the islet are intensely stained and when such a preparation is studied under an apochromatic immersion lens it is seen that the stain is contained exclusively in the small granules described above, which are imbedded in a colorless and apparently structureless protoplasm. When such preparations are exposed to the air, however, in the cells stained with janus green the mitochondrial granules also take up the stain though they are difficult to observe among the specific granulations of the cell.

Both in the fresh preparation and in those stained in neutral red the small granules exhibit brownian movement.

If fresh preparations of the islet cells stained with neutral red are kept under observation for one-half to one hour in isotonic salt solution, a change in the structure of the cell may be observed, the protoplasm then becomes coarsely vacuolated, or alveolar in structure, the granules disposing themselves in the partitions between the alveolae.

In studying fresh preparations of the islet cell the method of obtaining sections by freezing must be avoided, for the granules disintegrate when frozen and in the neutral red preparations the dye is dispersed throughout the protoplasm.

An interesting feature of the preparations stained by neutral red intra vitam is that owing to the small size of the granules it is possible to observe in the still living cell the outline of the canals of Holmgren, which appear as colorless spaces among the deeply stained granules.

The examination of these preparations also dispels the idea that the cells of the islet constitute a syncytium, for not only can the outlines of the individual cells be seen perfectly in the intact islet, but a slight pressure on the cover glass suffices to separate the cells from one another. It is thus possible, without teasing, easily to obtain large numbers of single islet cells for high power study.

In the single cells isolated by the methods just described the granules show frequently an unequal distribution in different parts of the cell. For example, many cells of elongated shape show the granules concentrated in each end of the cell while the intermediate area may have few granules. Others show such a concentration of granules in one end of the cell only. Many cells show the granules uniformly crowded with granules in all parts. On the contrary a few cells may show no granules at all. Whether these differences are due to different stages of physiological activity or not, I am unable to say, for I have not been able to influence the number of the granules by any experimental method, but the concentration in the one end of the cell is explained by the fact that the fixed preparations show that this is the case in the end of the cell which abuts on the capillary, while in the opposite end of the cell the presence of the canals of Holmgren in part explains the poverty in granules.

In fresh preparations it has not been possible for me to distinguish the granules of the *A* cells from those of the *B* cells, nor have I found in fresh preparations mounted with proper precautions the differences of refractive index of the granules described by Languesse. The granules of the *A* cells, however, hold the janus green longer than those of the *B* cells, so that, after a time, these stand out as blue stained cells on a background of red produced by the reduction of the janus green in the *B* cells.

In proceeding to the study of the islet cells by methods of fixation, sectioning and staining it was necessary to secure the preservation of the islet cell granules in both types of cells, and to stain them differentially. Lane showed how the granules of the *B* cell and those of the *A* cell could be brought out in different preparations, but for experimental work it was necessary to be able to identify them with certainty in the same preparation. I have found that this object may be accomplished with ease in preparations fixed in Lane's chrome sublimate material by staining in acid fuchsin after the neutral gentian. In these preparations the granules of the *A* cells are stained red, those of the *B* cells violet. Also, in preparations fixed in acetic osmic bichromate and stained in anilin acid fuchsin and differentiated in methyl green, the *A* cells stain deeply red, the *B* cells green. Material fixed in this way also stains differentially in safranin acid violet, the *B* granules staining red the *A* granules violet. For demonstrating the *A* granules alone, I have found, fixation in acetic osmic bichromate, and staining in acid fuchsin with differentiation in picric acid best. In these preparations, the *B* granules remain unstained while the *A* cells stain intensely red. For the *B* cells alone the method recommended by Lane is best.

For the nuclei and chromophile substances I have used toluidin blue and other basic dyes without counterstain.

In preparations in which the granules of the islet cells are not preserved or in which they remain unstained, the protoplasm exhibits an extremely fine alveolar structure, which I believe to be due to the same cause which produces the coarse alveolar structure in the inner zone of the acinus cell, namely, the alveolae represent granule-holding spaces. In badly fixed material a coarser alveolar structure may be seen which is due to post mortem changes in the cell which I have described above as appearing slowly in cells studied in salt solution.

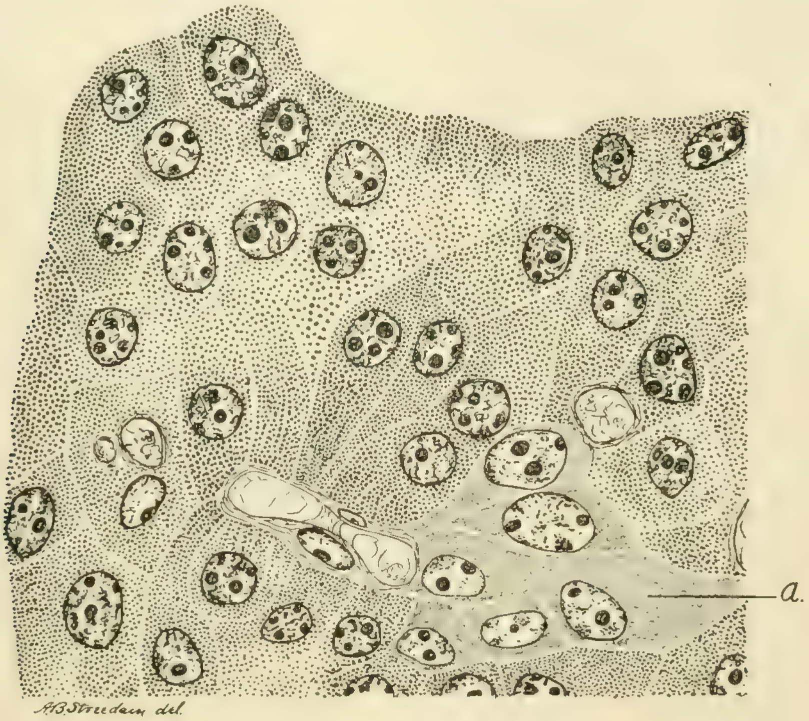

Fig. 12 Small portion of an islet of Langerhans of the guinea pig. Shows *B* cells filled with fine granules, and *A* cells stained diffusely; *a*, *A* cells. × 1066.

When the specific granules of the cells are stained they present the appearance shown in fig. 12. The protoplasm is studded with fine granules, which are imbedded in an apparently homogeneous ground substance.

The mitochondria of the islet cells may also be seen in the preparations fixed in acetic osmic bichromate and stained with acid fuchsin, etc., for though the granules of the cell are stained by this method they remain sufficiently transparent so that one can see the more intensely stained mitochondria among them. The latter differ markedly from the mitochondria of the acinus cells though they have the same staining characters. In the islet cells the mitochondria is in the form of delicate filaments and granules scattered throughout the cell, though there is often a slight concentration of them in the base of the cell near the blood-capillary.

The canals of Holmgren resemble those of the acinus cell both in topography and location for, while at first sight it seems as if the networks were located indifferently on the capillary or other side of the nucleus, when a column of cells composed of two layers is found cut through the center, the canals are invariably found on the side of the nucleus farthest from the capillary. In the islet cells the canals form a more open network than in the acinus cell, and processes more frequently extend around the nucleus in the direction of the capillary.

The two kinds of cells of the islet are distinguished from one another by the staining properties of their granules and by nuclear characters. The former differences I have already indicated. The nuclei of the *A* cells are oval in outline and much less rich in chromatin than the acinus cells and *B* cells. The nuclei of the *B* cells are spherical or slightly oval, and contain large chromatin granules. Both *A* and *B* cells lack the large eosinophile nucleolus which the acinus cell possesses, though smaller eosinophile bodies may be often observed.

When studying the cells of the islet by methods which demonstrated the one type to the exclusion of the other, as for example, by the methods of Lane it seemed as though the two types described included all of the cells of the islet. As soon, however, as it became possible to stain the two types differentially in the same preparation it became apparent that there were, in the islet, cells which contained neither *A* granules or *B* granules. In the acetic osmic bichromate preparations, stained as described above, these cells stand out as clear cells with numerous rather coarse mitochondrial filaments. Their nuclei resemble those of the *A* cell but are usually more elongated. In the *A* cells also the granular content is much more variable than in the *B* cells, and one may find *A* cells which differ from the clear cells just described only in the fact that they contain a few fuchsinophile granule, and all gradations of granule content up to cells which are crowded with granules. This suggests a possible source for the *A* cells, namely that they may arise by differentiation of the clear cells which are included in the islet from the time of its formation.

In point of frequency the *B* cell far exceeds the other two, forming the major part of the bulk of all the islets. Many of the smallest islets consist of *B* cells with a few of the clear cells described above (figs. 5 and 9). This is true of the small islets originating from the duct which contain no *A* cells, though the larger islets of this group contain large numbers of them. The *A* cells and clear cells are not confined to any particular portion of the islet as supposed by some, but occur in the interior as well as on the surface (fig. 13).

Whether the two main types of islet cells are developmental stages of a single element or not, it is difficult to determine. The suggestion of Tshassonikow ('06) that the *A* cells were intermediate in character between the cells of the acinus and the *B* cells, I believe to be untenable for the following reasons: first, I have shown that they occur in large numbers in the large interstitial islets which have developed from the ducts without passing through an acinus phase; second, they are located in large numbers in the interior of the islet as well as on the surface where one might expect to find transitional elements; third, they are present in large numbers in the acini of new born guinea pigs unassociated with *B* cells, whence they disappear within the first week without a proportionate increase of *B* cells; finally the assumption that they are intermediate between acinus cells and *B* cells is based on another assumption that they present intermediate characters between these two types which is not true. As a matter of fact, *A* cells are as far different from acinus cells as are *B* cells, as Lane has clearly shown.

A second possibility that they are more advanced stages in the history of the islet cell than the *B* cell has more facts to support it, for I have shown that the small islets associated with the duct system contain at first no *A* cells, while the large islets contain them in large numbers. They must either have been formed from the *B* cells or from the undifferentiated cells which also form a part of the small islets. Unquestioned transitions between the *B* cells and the *A* cells I have not been able to find in my preparations, though, on account of the complex arrangement of the cell columns in the islet it is not difficult to find cases where a *A* cell is covered by a thin section of a *B* cell and so appears at

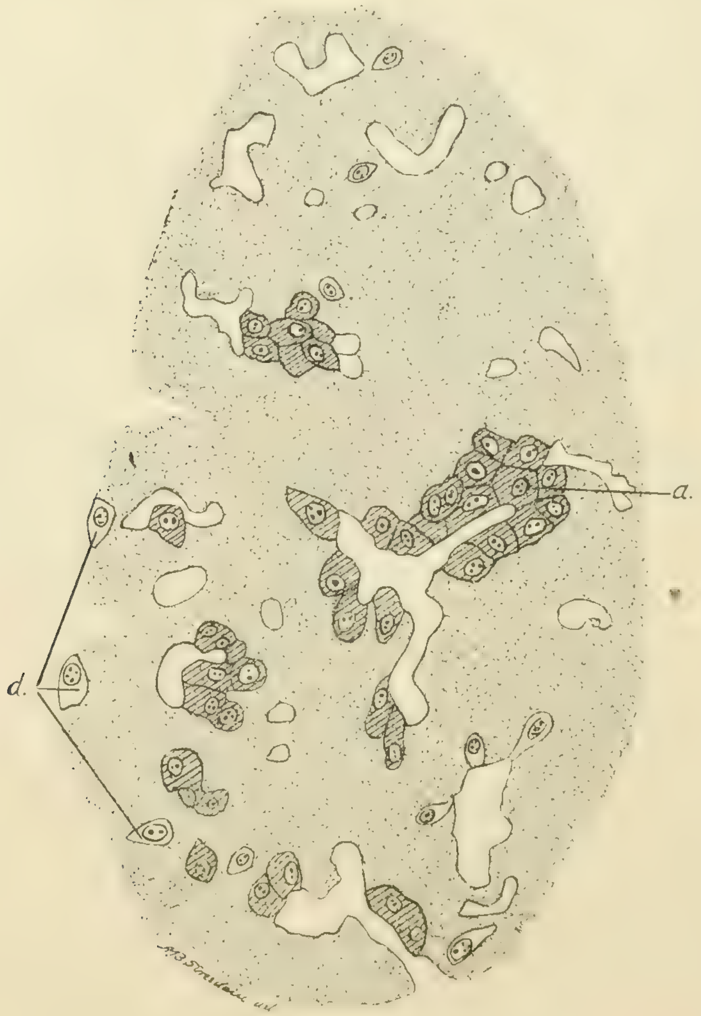

Fig. 13 An interstitial islet of the guinea pig showing distribution of the *A* cells, *B* cells and undifferentiated cells in the middle section of a series. Acetic osmic bichromate preparation stained in anilin acid fuchsin and methyl green; *a*, *A* cells; *d*, undifferentiated cells. The general tint indicates the mass of *B* cells. × 334.

first sight to contain both sorts of granules. On the contrary, there are abundant examples of cells which might be fairly considered as intermediate between the clear cells of the islet and the *A* cells. Accordingly, as far as my evidence goes I am obliged to conclude that the two types of islet cell are independent and that each develops from an undifferentiated duct cell.

3. *The structure of the epithelium of the intralovular ducts and the centroacinous cells*

The third type of epithelial cell involved in the discussion of transitions between islets and acini is the epithelial cell of the small duct. A description of this type has already been given in discussing the structure of the small tubules which connect the ducts with one another and which give rise to interstitial islets. Here, it is simply necessary to emphasize the differences between the cells of the ducts and those of the acini and islets.

From the acinus cell the duct cell differs in the fact that it possesses neither basal basophile substance nor zymogen granules, and also by the fact that it possesses an elongated oval nucleus which, by comparison with the acinus cell, is relatively poor in chromatin.

From the islet cells of both types the duct cell differs in the fact that it is totally devoid of the characteristic granules of the islet cells.

The mitochondria of the duct cell is unusually abundant, which is quite in accord with what we would expect in view of the high developmental potency of the duct cell. In fact the cells of the smaller ducts when stained for mitochondria, resemble closely the embryonic cells described by Meves ('08), inasmuch as the only formed elements visible in them are thick mitochondrial rods (chondrioconts).

The cells of the terminal ductules including the centroacinus cells are richer in fuchsinophile bodies than the cells of the larger intralobular ducts (fig. 14). Much of this fuchsinophile material is in the form of the characteristic mitochondrial rods, but, in addition there are irregular spherical or angular granules present concerning the interpretation of which I am in doubt. Possibly they represent in part a secretion antecedent.

TRANSITIONS BETWEEN ACINUS CELLS AND ISLET CELLS

Having now defined the islet cell as an element containing granules which are peculiar to each type of cell composing the islet, and which occur in no other cell of the pancreas, it is possible to discuss on this basis the question of what should be accepted as a scientific demonstration of cells which are intermediate in type between islet cells and acinus cells.

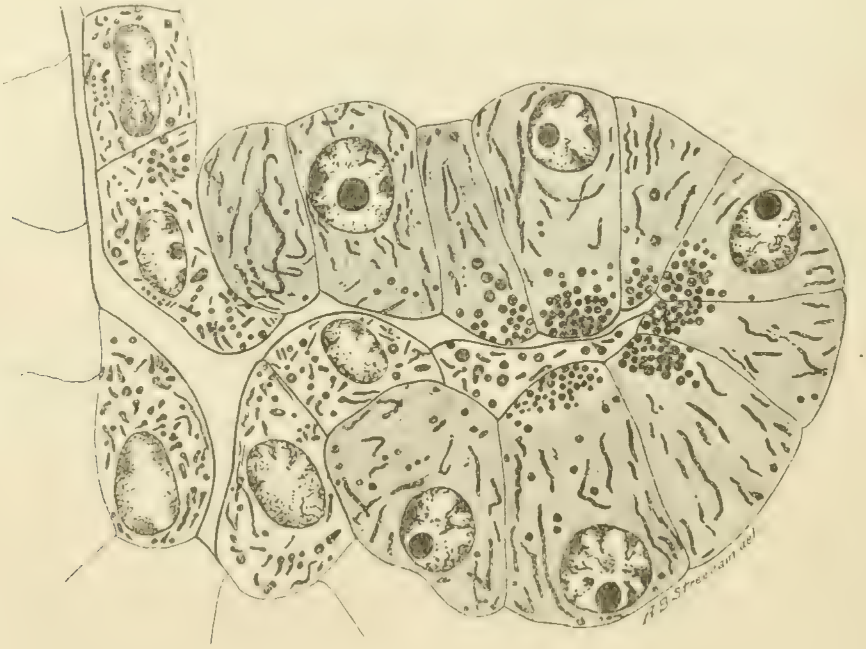

Fig. 14 Section of an acinus of the pancreas of the guinea pig, showing centro-acinous cells with mitochondria and fuchsinophile bodies. In the acinus cells may be seen, zymogen granules, and long mitochondrial filaments. Acetic osmic bichromate, anilin acid fuchsin, methyl green. × 1555.

It has been shown that the acinus cells have two constituents which are not present in the islet cell, namely zymogen granules and basophile substance, and also that each type of islet cell contains granules which are peculiar to itself and which are not present in the cells of the acini. It has also been shown that the duct cell contains neither the specific constituents of the acinus cell nor those of either islet cell. The mitochondria, moreover, while present in all four types have characters of their own for each type.

The question we now have to consider is: If a cell of an acinus were being transformed into an islet cell, through what stages would it be expected to go, and conversely if an islet cell were being transformed into an acinus cell what would be the phases of this transformation? I think it is clear, that we must consider all phases of this change before we decide lightly that it is a change of one element into another, that is, we must show not only the phases of the disappearance of the specific elements at one end of the series, but also the appearance of the specific elements at the other end. The number of stages which could be expected in this process would depend to a large extent on the rapidity with which the change proceeded, but it is reasonable to suppose that this change could not go on with too great a rapidity to permit the discovery of intermediate phases, because the anatomical construction of an islet, which does not contain centroacinous cells and which has a peculiar blood supply, necessitates a considerable degree of anatomical rearrangement in constructing islets out of acini.

It seems to me that to be a satisfactory evidence that this change of acinus into islets, or *vice versa,* is going on, one of the following possibilities would require to be fulfilled:

1. It should be possible to find intermediate cells which contain at one and the same time islet granules and one of the constituents of the acinus cell, that is, zymogen or chromophile substance; or

2. It should be possible to find a graded series showing the gradual disappearance of the characteristic constituents of the acinus cells, and, step by step, the appearance of the granules which are characteristic of the islet cells; or

3. It should be possible to find acini containing well defined centroacinous cells but with islet cells instead of acinus cells.

Failing transitions which fulfil one of these requirements, we would be obliged to fall back upon less satisfactory evidence of an indirect sort to prove transformation. It is conceivable that the process of transformation is begun by the obliteration of the lumen of the acinus. In this case we should find acini in continuity with islets but detached from their ducts. Similarly, if we can show that there is a quantitative variation of the reciprocal amounts of islet and acinus under different experimental conditions, we have in that fact satisfactory evidence that one tissue is increasing at the expense of the other.

We may now proceed to discuss the transitions which have been described by various authors as indicating a transformation of one tissue into the other.

The transitions described by Dale ('05), and those described by Vincent and Thompson ('07) are of the same sort and may be considered together. The conception which Dale had of the structure of an islet cell is well indicated in the statement that "occasionally, but rarely in the resting gland, an islet may be found, which is apparently continuous with the epithelium of a ductule, and the similarity of the islet cells to those of the epithelium is then very obvious." This, it need hardly be mentioned, is a negative similarity and does not necessarily mean any more than would a similar comparison of the islet cells and the epithelium of the bile duct. Again, he says:

> With a high power many of the islets show a division of the cells, by intervening connective tissue and blood capillaries into packets which have a shape strikingly similar to that of the secretory alveoli. In such, an outer layer of large cells can be distinguished from an inner layer of smaller cells exactly like centroacinary cells. The appearance in fact is exactly that of a group of alveoli in which the secreting cells have lost their characteristic basal basophile staining and zymogen granules, their nuclei having become more centrally placed, and assimilated to those of the centroacinary cells, and in which the lumen has been obliterated by a falling together of the cells.

The deceptive alveolar arrangement here is due to a section across a cell column of the islet with *A* cells surrounding *B* cells. In another place he sums up the evidence in favor of the theory of transformation as follows:

> The unequal distribution of the staining reaction, so that an isolated cell or part of a cell shows the basophile reaction, which fades away towards its edge; the presence of cells which have no basophile reaction, but retain a few eosinophile granules, which appear to be undergoing solution; the faint trace of an alveolar outline, like the shadow of a structure which is being lost. . . .

In discussing the effect of prolonged secretin stimulation Dale adds:

> It can be seen that, apart from the large masses of definite islet tissue such as the low power shows, a large proportion of the remaining alveoli show partial change into what must now be called the islet condition, some of the cells having lost their normal staining properties and having become assimilated to the centroacinary cells.

Similarly Vincent and Thompson ('07) in describing the islets of the guinea pig say: "Transitions are so common that, in the majority of the islets, it is quite impossible to define their limits under a high power, since the two tissues fade gradually into each other. There is a comparatively wide zone of cells which partake in a varying degrees of the characters of zymogenous and islet cell." Describing the islets of the dog the same authors say: "There are all transitions to be found between the most strongly granulated of alveolar cells and the clearest of islet cells."

It is quite apparent that the three authors in question have considered in this connection only the loss of the basophile substance and of zymogen granules, and that they were quite willing to identify as an islet cell any cell not in a duct which had neither zymogen nor basophile substance. There is no indication whatever that they have given any consideration to the specific granules of the islet cells, which Dale says he was unable to find, and which Vincent and Thompson mention, but ignore when discussing transitions. Their work is based on the incorrect assumption that islet cells do not differ from duct cells and their whole case falls to the ground when it is shown that the assumption is unsound.

If Vincent and Thompson had employed the methods of Tschassonikow ('06) for the study of the islets of the guinea pig, their difficulties in defining the actual limits of the islets under the microscope would have vanished, for as a matter of fact the transition zone which they describe does not exist, as may be seen from my figures of the neutral red preparations.

The question, however, still remains whether the cells described by Dale as produced from acinus cells by long stimulation with secretin are islet cells or not. If they are islet cells they should show the characteristic granules of islet cell by vital staining with neutral red, or in preparations by methods which have been found to be adequate. Earlier in this paper, I have answered this question by showing that the assumption of Dale that secretin increases the number of islets is unwarranted by the facts. The examination of fixed material from guinea pigs and toads which have been long stimulated by secretin confirms this opinion for in the sections of this material I find the cells described by Dale, but when stained by methods which bring out the islet granules they are found to contain none. They are, therefore, not islet cells at all but simply exhausted acinus cells.

Dale and Vincent and Thompson give figures which are designed to show transformation of acinus tissue into islet tissue, by showing acini on the edge of islets which are apparently merely half acini, or acini in the midst of islet tissue. These appearances become clear when one sees the islets of the dog, toad, etc., *in toto*, in preparations stained intra vitam with janus green. In these preparations, it may be seen that the peculiarities of shape of the islets are sufficient to account for any relation of islet tissue to acinus tissue in sections. Islets even occur which are ring-shape, a section through the middle of which would show islet tissue completely surrounding acini, though a series of sections would quickly demonstrate the fact that these acini are in reality continuous with other acini.

Tschassonikow ('06) described the *A* cells in the islet as transitions, between the acinus cells and the *B* cells. On what grounds he came to this conclusion I do not know, because his main publication on this topic is unfortunately unavailable to me. This conclusion I have already discussed in connection with the remarks concerning the relation of the several types of islet cells to one another, and have shown that it is untenable. If we accept the *A* cells as transitions, then, we are entitled to ask where may be the transitions between acinus cells and *A* cells for the latter are as different from the acinus cells as are the *B* cells, since they contain neither zymogen granules nor basophile substance, and since their mitochondria are different from those of the acinus cell. I fear that the idea, that *A* cells are transitions, is merely a hasty assumption based on the fact that these cells sometimes occur on the surface of the islet and sometimes are interposed between the acinus cells and the islet cells. Laguesse ('06–'08) in his summary of the literature supports this idea of the *A* cells but in his latest paper appears to have abandoned it, because he describes there a different sort of transitional element, and attempts to realize the logical necessities of the transition as I have indicated them above.

Mankowski studying preparations of the pancreas of the guinea pig fixed in Flemming's fluid found cells which he regarded as transitions. These require careful consideration, for Mankowski ('01) demonstrated the granules in the islet cell, and found similar granules in certain acini which were alongside of the islet. Laguesse ('08) also described similar granules in the base of the acinus cell of the human pancreas, which he regarded as evidence of the assumption, by the acinus cell, of a new internal secretory function.

These acinus cells containing small granules I have studied with great care, because if the granules were really similar to islet granules, then it might be claimed with justice that the first of the criteria of real transitions which I have laid down, had been established and that these cells were in reality transitions. As a result of this study I have found that these granules are not islet granules at all but that they result from a degeneration of the basophile material of the acinus cells. In some guinea pigs this granular condition of the acinus cells is so abundant that practically every cell in the pancreas may show some trace of it. In such pancreases, it is very easy to study the process of formation of the granules. The process begins by the appearance of a few of the granules in the base of the cell. As they increase in number the basophile material diminishes. Later the zymogen granules disappear, but there is an intermediate stage where the cell contains a few zymogen granules but all of the basophile material of the base of the cell is replaced by minute granules (fig. 15). Associated with the disappearance of the basophile material there is a profound modification of the mitochondrial filaments When the base of the cell is well filled with the tiny granules but some zymogen is still present the mitochondria of the affected cells are seen to differ from the surrounding normal cells, in the fact that the rods are shorter and thicker and show a tendency to round up into spheres As the process advances the mitochondria assumes the condition of large round spherules which gradually diminish in size and ultimately disappear. Meanwhile, all of the zymogen has disappeared from the cell and the number of small granules has so increased that they fill the entire cell.

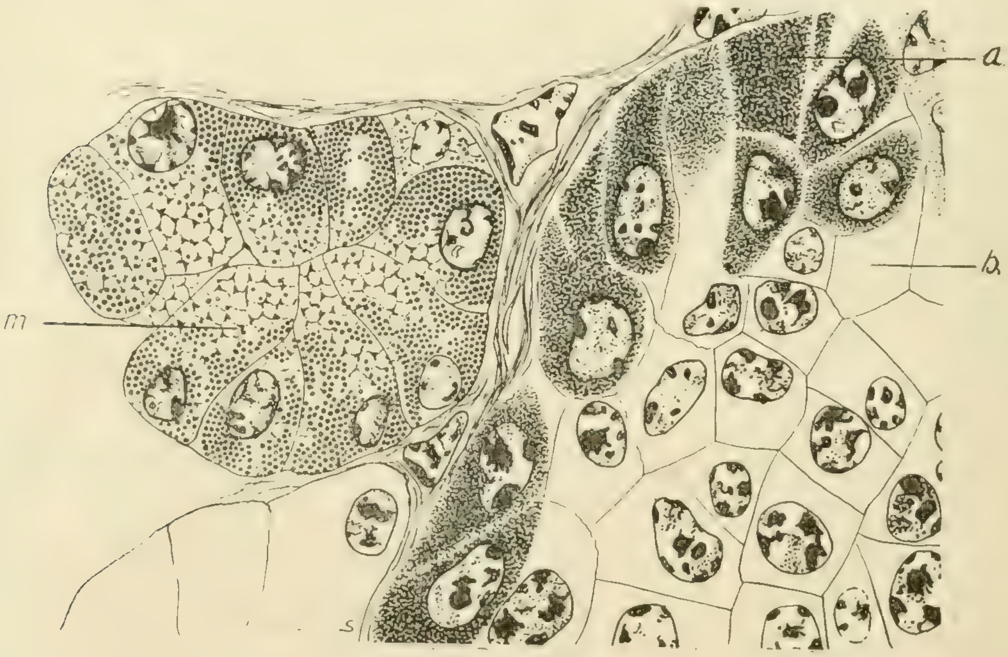

Fig. 15 A pancreatic acinus from the guinea pig, showing cells containing Mankowski granules, and alongside a portion of an islet with *A* cells and *B* cells differentially stained; *m*, Mankowski granules; *a*, *A* cells of islet; *b*, *B* cells of islet. Hermann's fluid, neutral gentian.

This change in the pancreas can be easily recognized in the fresh tissue, for the granules have a high refractive index, and so stand out as cloudy cells among the otherwise transparent acinus cells. The granules are larger than those of the islet cells and more refractive.

That these granules are not the same as islet cell granules may be shown in many ways. For example, they do not stain in either neutral red or janus green used intra vitam. Furthermore, they are easily preserved in any of the ordinary fixing fluids and stain readily in gentian violet or safranin, whatever the fixation. It is thus possible by a suitable choice of fixing agent and stain to retain the Mankowski granules, and to eliminate in turn the *A* granules and the *B* granules, and even the zymogen granules.

Accordingly, we must conclude, that Mankowski's identification of these granules as islet granules was based on a superficial resemblance to one another of two things which are really not similar.

Laguesse ('06–08) identified the cells of the *A* type as cells of transition between the acinus cells and the *B* cells which he regarded as the definitive islet type. He says: "Nous ajouterons encore. . . . que nous y voyons un stade de transition entre les cellules principales d'acini et les cellules d'îlot en periode d'état. Chez le cobaye notamment, nous ne recontrons qu'un petit nombre de ces cellules de deuxième variété, et nous les trouvons précisément aux points de continuité avec les acini, ou même isolées dans les acini voisins."

I have already discussed this conclusion and have shown that it is based on incorrect notions of the distribution of the *A* cells, which do not occur exclusively on the surface of the islet, and do occur in large numbers in islets which have no connection with acinus tissue. The *A* cells which Laguesse supposed to occur in the neighboring acini are probably acinus cells undergoing the Mankowski degeneration, for in the guinea pig the *A* cells only occur in large numbers in the acini in the first week of life.

The arguments advanced by Laguesse in favor of the theory of transformation, I have examined with great care in his voluminous writings on this topic, and with the exceptions which I shall discuss later, they seem to me to prove nothing more than continuity of islet tissue with acini and with ducts. His latest paper on the conditions in the human pancreas is largely devoted to this proof of continuity, and I have already indicated my full agreement with him in this respect. But does continuity necessarily mean that the islets are growing at the expense of the acini, or the acini at the expense of the islets? I think not, for if we admit this contention for the pancreas we must also admit it for other tissues, and conclude, for example, that the demilunes of the mucous glands are derived from mucous cells or *vice versa*, and that the parietal cells are derived from the chief cells of the gastric glands.

When we begin to search in Laguesse's articles for the evidence of transformation beyond the fact of continuity, we find that such evidence is very meager and not always consistent. For example, in one place he describes the *A* cells of the islets as transitional cells, and in his late article on the human pancreas describes another case of direct transformation of acinus cells into *B* cells with an intermediate type which is similar in some respects to both.

In his general résumé of the literature ('06–'08) he gives in figs. 67 and 68, and in the accompanying text, illustrations and descriptions of the growth of an islet in the human pancreas at the expense of neighboring acini with which it is continuous. In these figures he depicts, around the islet, on each side, groups of cells which connect it with the surrounding acini, which, he says, possess intermediate characters. I have examined these figures carefully, and studied his description, but have failed to find any evidence that these cells are not, as they seem, in part duct cells, and in part acinus cells cut tangentially. As the material was fixed in alcohol any fine cytologic differentiation was impossible. Similarly, fig. 69 in the same article is, it is true, an abnormal acinus, but the evidence that it has been derived from an islet is wholly lacking.

That the fact of continuity, and of intermixture of the two tissues has had great influence in Laguesse's mind is indicated by the following extract from his discussion of these conditions in ophidians:

> De deux choses l'une, ou cet engrènement, ce mélange est l'indice d'une transformation de l'un des deux tissus en l'autre, ou bien il faut le supposer persistant depuis l'époque lointaine du développement embryonnaire. Peut-on encore admettre cette dernière supposition en ayant sous les yeux les figures précédemment données (figs. 63 and 65 showing continuity)? Pour nous, cela nous est impossible. Nous avons une toute autre conception de la vie; la vie, c'est le mouvement; la vie d'un groupe celluleire c'est le changement incessant de sa forme et la renouvellement de ses molécules.

Even if we admit this conception of cell life it is a far cry from the simple renewal of the cell's molecules to the complete transformation of it into another type. One cannot help wondering however, whither this conception of cell life would lead Laguesse if he should attempt to apply it to the case of the gastric glands with their intermixture of chief and parietal cells, or to the intestinal glands, or to the nervous system. However, Laguesse's two alternatives do not exhaust the possibilities of the case, as he supposes, for it is possible if the cells are not really persistent throughout life, which we do not know, that both acinus cells and islet cells of such a mixed group, after a certain period of functioning, disappear and are replaced by new elements which grow out from the relatively undifferentiated but highly potent epithelium of the duct. This possibility is well illustrated by the observations of Gontier de la Roche ('02) who showed that after the disappearance of the acinus tissue, resulting from occlusion of the pancreatic duct, both new acini and new islets were formed from the duct system.

In his latest paper Laguesse ('10) has described two conditions which are more significant than the foregoing, and which, if confirmed, under proper precautions would go a long way to establish his claim that acini may be converted into islets, though he would still be far from proving that this process is of common occurrence in the pancreas. In his article on the relation of islets to acini in man he describes a cell which is interposed between islet cells on the one side and acinus cells on the other and which partakes of the structure and staining reactions of both. This cell is from a preparation fixed in acetic sublimate and stained in safranin picric acid and naphthol black. The cell shows on the side next the islet faintly indicated islet characters, and on the other well defined acinus characters. This if a little more definite, is the kind of transition which I have sought with great care in my preparations, but without success, for when I thought at first that I had found such a cell, I have found invariably, on closer study, that it was simply a case of overlapping of the two cells. This, I suspect, is also the case in Laguesse's preparation. For although he is careful to say that no islet cells overlie this cell in the neighboring sections, yet the appearance would be well explained if one supposed that the adjacent islet and acinus cells had an oblique surface of contact so that a thin sector of the islet cell was included in the same field of view as the acinus cell. Overlapping of this sort is very confusing, but easy enough to differentiate if the granules of the islet and acinus cells are sufficiently well stained.

The second statement of Laguesse which would carry conviction if sufficiently well established is to the effect that, on the surface of the islets in the human pancreas, he finds remains of acini, which have no connection with the duct system, and which are included under the same basement membrane as islet cells. The last half of this statement, is of course merely a restatement of the fact of continuity, for continuity involves continuity under the same basement membrane. The acini in question, according to Laguesse, form cap-like masses over the ends of columns of islet tissue which terminate on the surface of the islet. In discussing this condition he quotes Dewitt ('07) as having found a similar condition in the rabbit, which she explained by assuming that the embryonic connections of the islets and acini had persisted. Laguesse criticises this conclusion on the ground that experiments with duct ligation showed that acini cut off from their duct connection, rapidly lost their zymogen. On examining Dewitt's article, however, I find that Laguesse has misunderstood Dewitt's description, for her intention is to describe continuity between islet and acinus tissue without admitting that the latter is disconnected from the duct. Laguesse, in this discussion has furnished an argument against his own conclusion that the acini observed by him on the periphery of the islet are separated from their duct, for if this were true, according to his own statement, zymogen should not be present in the acinus cells. It is well to remember also in this connection that it is frequently impossible to see the lumen even in acini far removed from the islets, because the cells fall together in fixation and the lumen is thus obliterated.

Laguesse's figures on plates 1 and 2 ('10) giving a complete series through an islet and the surrounding tissue, I have examined carefully, and while in some places single sections seem to show isolated groups of acinus cells in contact with islet cells and detached from the neighboring acinus tissue, following the series through shows that every acinus cell is connected with the duct by other acinus cells or by centroacinous cells.

In my own preparations of the pancreas of the guinea pig I have found abundant examples of the same sort as represented in these figures of Laguesse but I have invariably in these cases been able to trace the connection of these half acini with the duct in series. The examination of preparations in which the duct cells are stained intra vitam by methylene blue confirms this conclusion, for in such preparations large numbers of acini may be found which are separated from islet cells only by a group of centroacinous cells surrounding a lumen, or half acini bordering a cavity which is bounded on the other side by islet cells. Accordingly I am of the opinion that Laguesse has not sufficiently well established the principal feature of this case, namely, the separation of the half acini from the ducts.

An examination, then, of the facts advanced in support of the statement that transitions exist between islet cells and acinus cells reveals the fact that some of these supposed transitions had their origin in an imperfect conception of the islet cell. This is true of the transitions of Dale, Vincent and Thompson, who identified the islet cells by negative characters and thought that they were not essentially different from duct cells

The idea that the *A* cells of the islet are transitions, supported by Tschassonikow and for a time by Laguesse, has to commend it only the fact that *A* cells are different from *B* cells and that they sometimes occur on the surface of the islets, though not exclusively so. On the other hand they occur equally abundantly in the interstitial islets where there can be no question of their origin from acinus cells.

The transitions of Mankowsky are shown by careful investigation to be in reality degenerations, and the arguments of Laguesse as far as the pancreas of the adult is concerned are reduced to evidences of continuity of the two tissues which certainly do not carry the implication that the one has been derived from the other.

Furthermore, a quantitative examination of the normal content of islets in the pancreas of the guinea pig, and of that of guinea pigs which have been subjected to secretin stimulation or inanition, establishes the fact that the number of islets is not influenced by these conditions.

Accordingly we must conclude that the possibility of the transformation of acinus tissue into islet tissue or conversely of islet tissue into acinus tissue has not a single well established fact to support it.

It must be remembered, however, that our results do not justify us in drawing the conclusion that this transformation is impossible, though they make it appear improbable, for we cannot draw conclusions concerning the fundamental potencies of the cells from negative results of experiments. The latent potency to form islet cells may be present in the acinus cells notwithstanding our negative results, and at any moment an experiment may be devised which will enable us to call forth these latent energies. A case of this sort is afforded by the observations of Cade ('01) and Harvey ('07) on the gastric glands of the dog. They found that after gastroenterostomy the chief cells of the fundus glands near the site of operation underwent a change of function and assumed the appearance, and staining properties of the mucous cells which are normally present only in the neck of the gland. Harvey ('07) found that after a certain period these cells recovered their zymogenic function showing that the change was not a degeneration. Here, then, we have the case of a group of cells preserving intact the power to form mucous cells, though under normal circumstances this power is never expressed. In the same way, it is possible to conceive of an experiment which will call forth the latent potencies of the acinus cells and islet cells, and cause either for a time at least, to assume the characters and functions of the other type.

I am the more willing to admit this possibility, because of the fact that the study of the relation of the system of branching tubules, which I have demonstrated, to the new formation of acini and islets, has convinced me that the cells of these tubules as well as the cylindrical cells of the ducts from which they arise, are capable of developing into new islets or new acini by mitotic division and differentiation. These cells thus possess at least three developmental potencies, namely they may form acinus cells, *A* cells, or *B* cells. This conclusion is also supported by the observations of Laguesse and Gontier de la Roche on the changes in the pancreas after ligation of the duct. They found that the cells of the tubular system to which the pancreas is reduced after this operation were capable of regenerating new acini, or new islets containing both types of cells.

The fundamental question then that we are required to answer is whether the fully developed acinus cells or islet cells retain any of the potencies which resided in the cells from which they were derived, and if not, at what period in the cytomorphosis of these cells are these potencies lost. Only experiments which yield positive results can give us any light on this question, for it is possible, even though we never find in the normal pancreas, acinus cells developing into islet cells, or *vice versa*, that the acinus cells nevertheless possess islet potency, and will bring it to expression if the necessary functional demand is made.

In view of the results obtained by Harvey ('07) and Cade ('01) which have been quoted above I am inclined to think that this view of the developmental potencies of the pancreatic cell will prove to be correct, even though under normal conditions of equilibrium and in view of the regulatory powers which reside in the duct epithelium, these potencies may never under normal conditions be brought to expression.

While admitting the possibility of the transformation of acinus tissue into islet tissue, though insisting that at present we have no actual demonstration of its occurrence, it seems to me that we must also insist that the facts indicate that the normal regulation of islet content in the pancreas is not by this method, and that Laguesse's theory of balance which supposes that every acinus in the pancreas may at some phase in its history pass through an islet phase, is untenable. For while Laguesse admits that islets are in direct continuity with ducts, according to his theory, if I have correctly interpreted his explanation, they have this connection only because they are formed from acini. Accordingly he is obliged to explain Dewitt's observation of the occurrence of islets in the interstitial tissue of the guinea pig unassociated with acinus tissue, by the assumption that her observation was incomplete, and that she would have found, if she had studied complete series, small groups of acini attached. I have shown, however, that Dewitt's observation is correct, and that there occur all along the duct in the guinea pig large numbers of islets which are directly connected to the duct, but have no connection with acinus tissue. This is also true for the dog, cat, rat, and rabbit. The series of stages represented by these interstitial islets ranging from a single cell through all gradations of size leave no room for doubt that these islets are derived by direct growth from the ducts, and that they are in a certain sense permanent organs of the pancreas, for while it is probable that the cells of these organs go through a certain series of cytomorphic changes ending in their death, yet these islets have, in their duct connections, and in the undifferentiated cells which form a part of them, the materials for their own regeneration and repair.

In discussing the large islets of the splenic end of the pancreas in reptiles for which he admits a certain degree of permanency, Laguesse explains this permanency by suggesting that it is only relative, and that it is possible that these islets while permanent as regards size and approximate location, may nevertheless be constantly changing their substance by the inclusion of new acinus tissue on one side while the islet may be changing into acinus on the other. Thus the islet is retained but its cellular material is ever changing. The question, however, is not what is possible, but what is true and I fail to find in Laguesse's discussion on this topic any evidence that this process is actually going on.

Another fact which it is impossible to reconcile with the hypothesis of balance of Laguesse is the fact that the islets of Langerhans are unequally distributed in the pancreatic lobule. It is well known, and is admitted by Laguesse, that the islets rarely occur at the surface of the lobule. If Laguesse's hypothesis were true, islets should be equally frequent in all parts of the lobule. Laguesse sees in this fact merely an indication that acini in the centre of the lobule have a greater tendency to undergo this change than those on the surface, but this is merely stating the facts in terms of his theory and is no explanation.

On the contrary, a very simple explanation of this predilection of islets for the central portions of lobules is possible when we consider how the interstitial islets take their origin, and how the intralobular islets are related to the ducts. If islets are originating throughout life from the ducts and if the intralobular islets are in direct continuity with the ducts as Laguesse and I have shown, is it not probable that the intralobular islets originate in the same way as the interlobular ones, namely from the duct epithelium? If this be true, then the islets would of necessity occur in the interior of the lobules, where the ducts from which they have arisen are located.

I am accordingly of the opinion that the normal regulation of islet content in the pancreas is by interstitial growth of pre-existing islets, and by the formation of new islets from the duct epithelium, and not at all by the formation of new islets out of acini.

This conclusion is in accord with the observations of Helly ('06) and Weichselbaum and Kyrle ('09) on the origin of the islets, and with the experimental work of Kyrle ('08) on the regulatory processes in the pancreas after resection of a portion of its substance.

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THE BI-LOBED FORM OF THE VENTRAL PANCREAS IN MAMMALS

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TWELVE FIGURES

Among some pig embryos sectioned for class use, the writer, in 1907, found one in which the intestine was nearly encircled by pancreatic tissue. This unusual condition was due to the presence of a large left lobe of the ventral pancreas. The specimen was referred to Dr. Thyng for further study, and he described it briefly in vol. 7 of this Journal (1908, p. 493). He suggested that the annular pancreas of man may have a similar origin. Quite apart from this possibility, which will be considered later, the condition in the pig is of interest because of its frequent occurrence, and because it represents a more symmetrical development of the ventral pancreas than is normal. The great variation in the ventral pancreas, even in its earliest stages, renders its description difficult, and accounts for the conflicting results of careful investigations.

It is generally agreed that as soon as the ventral pancreas becomes an easily recognized structure, it is a median ventral outgrowth from the ductus choledochus near its intestinal orifice. Preceding this stage, it is said to be represented by a pair of knob-like epithelial buds, situated one on either side of the hepatic diverticulum or primitive ductus choledochus. The paired stage is shown in fig. 1, from a pig embryo of 3.6 mm. It has not been previously recorded for the pig, but corresponds closely with the condition found in a sheep embryo of 4.5 mm. by Stoss and clearly figured for the same species by Choronshitzky. It has been observed in the rabbit, rat, guinea-pig and cat by Helly, and has been figured by Debeyre in a human embryo of 4.5 mm. In Debeyre's specimen, however, the buds are ill-defined.

In a pig embryo of 4.5 mm. (fig. 3), the ventral pancreas is a median mass of cells in the angle between the ductus choledochus and the intestine. On the left side, this mass connects with a lateral outgrowth (*x*) which extends through six sections, terminating below in the section figured. On the right side, there is a corresponding outgrowth extending through four sections, but it is separated by one section from the median mass. If these lateral outgrowths correspond to the buds shown in fig. 1, it appears that the median mass has arisen in the interval between the buds, on the lower or posterior side of the ductus choledochus. Frequently the median mass has been described as continuing upward on either side of the bile-duct, producing symmetrical lateral swellings. This condition was found by Jankelowitz in a human embryo of 4.9 mm. It was observed by Brachet in rabbit embryos of ten and one-half to eleven and one-half days and he described it as follows:

"The ventral pancreas is formed at the expense of a semicircular fold embracing the posterior circumference of the ductus choledochus and the adjacent parts of its lateral walls . . . . It really represents two outgrowths which have fused."

Hammar, in the following year (1897), described the ventral pancreas of the rabbit in the same way. He wrote:

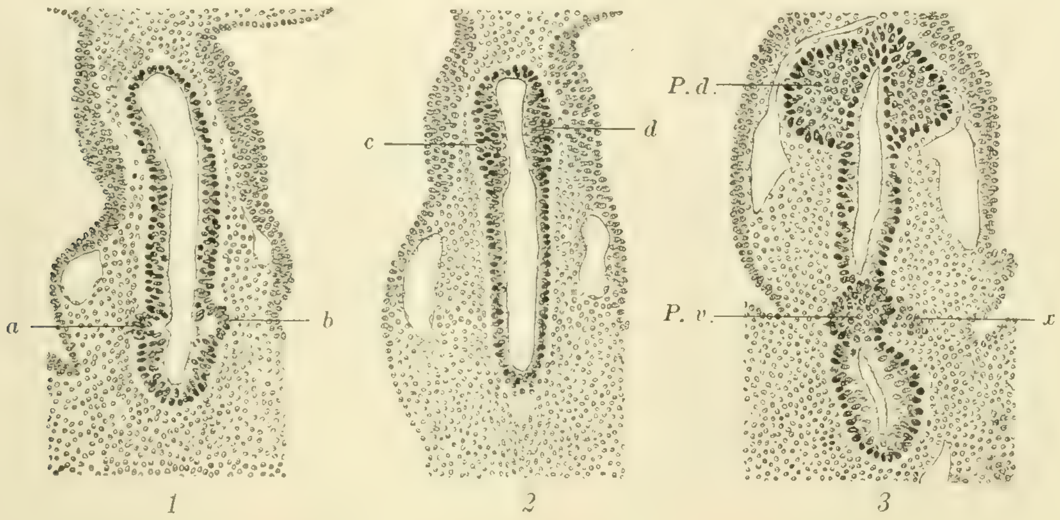
"It appears on the ductus choledochus as a thickening or outpocketing which semicircularly embraces the caudal and lateral surfaces of the duct. As soon as it projects freely, it appears as a single caudally directed outgrowth of the ductus choledochus." But he concluded that the description of the ventral pancreas as paired was "not applicable to the rabbit, dog and probably other mammals."

Choronshitzky (1900) described this condition in sheep embryos in almost the same terms, yet he regarded the ventral pancreas as paired.

"The caudal or ventral wall of the ductus choledochus, between the two ventral pancreatic buds, may be semicircularly thickened or outpocketed so that, to a certain extent, a connection is formed between these buds. . . . . In the next stage we see a single ventral pancreas which has arisen through the confluence of the two ventral pancreatic buds."

Helly (1901), after studying rabbit, rat, guinea-pig and cat embryos, concluded that the ventral pancreas is paired, but that its parts do not fuse. He writes as follows:

"The ventral buds develop from the lateral walls of the ductus choledochus. They arise quite separate from one another; in no case could I observe a fusion between them. The left ventral bud undergoes degeneration."



Figs. 1 and 2 Transverse sections of a pig embryo of 3.6 mm. (Harvard Embryological Collection, Series 1406, Sections 119 and 128). × 75 diam.

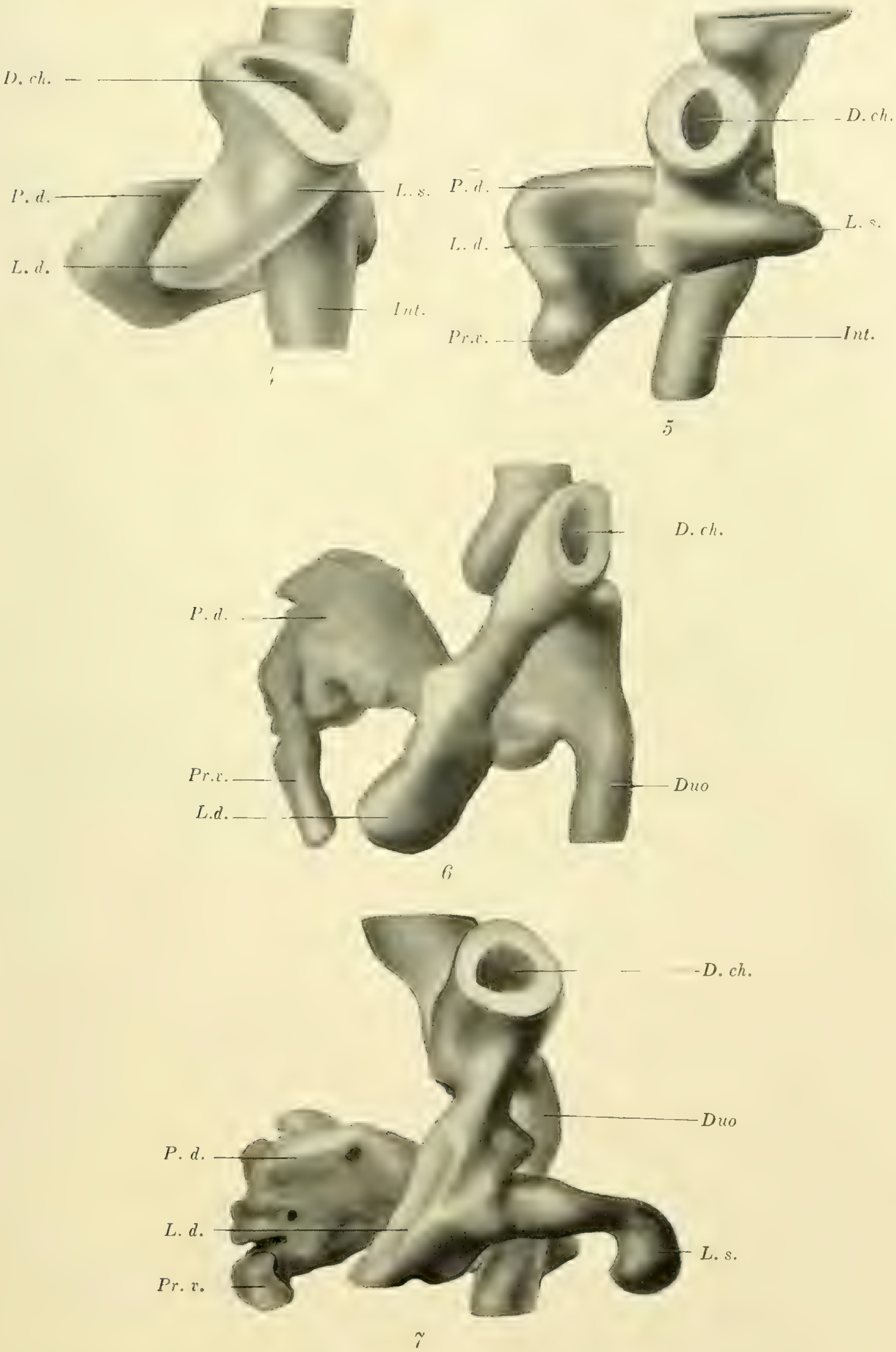
Fig. 3 Transverse section of a pig embryo of 4.5 mm. (H. E. C., Ser. 1404, Sect. 104). × 75 diam. *a, b,* buds which give rise to the ventral pancreas, *P. v.* *c, d,* plates which give rise to the dorsal pancreas, *P. d.* *x,* lateral proliferation.

In the Harvard Collection, there is rabbit embryo of eleven and one-half days (5.6 mm.) which appears to accord with Helly's description, since the median mass is directly continuous with a swelling on the right side of the bile duct, and the swelling on the left side is smaller and perhaps free from the ventral pancreas. But in two other rabbits of the same age, this relation is not seen; in them, the ventral pancreas appears to be exactly median, and it encroaches little, if at all, upon the sides of the bile duct. Moreover Helly's interpretation is not applicable to the pig embryo shown in fig. 3.

While the ventral pancreas of the pig is passing through its transient paired stage, the dorsal pancreas is also represented by a pair of epithelial proliferations (fig. 2, *c*, *d*). These are plate-like thickenings, and not round buds as in the case of the ventral pancreas. The plate on the right soon becomes larger than that on the left (fig. 3). At this stage (4.5 mm.), the dorsal wall of the intestine has become thickened and forms a part of the dorsal pancreas. Later, as in the 6.0 mm. embryo shown in fig. 11, the entire structure is an asymmetrical bi-lobed mass swinging toward the right. In embryos of 10 and 12 mm., the bi-lobed condition is generally clearly seen where the duct enters the duodenum (fig. 8), but anteriorly these lobes are lost in the compact mass which turns toward the right (fig. 7, *P. d.*).

The bi-lobed form of the dorsal pancreas of pig embryos has been recognized by Wlassow and Völker, but its paired origin has not been previously recorded. In the sheep, however, Stoss observed the parallel development of the dorsal and ventral pancreases, both of which he described as paired. Felix, Brachet, and Helly have denied the paired origin of the dorsal pancreas, which, indeed, is not apparent in the rabbit, or in other mammals in which the dorsal margin of the intestine is thickened from the first. But in pig embryos the parallelism is strikingly shown. Both the dorsal and ventral pancreases pass through a transient paired stage; both become median structures which are more or less bi-lobed, and both develop predominantly on the right side.

The ventral pancreas in pig embryos may extend wholly toward the right side, as seen in figs. 4 and 6, from embryos of 5.1 mm. and 10 to 12 mm. respectively. In the younger specimen, the upper part of the pancreas is subdivided by a groove into left-anterior and right-posterior divisions. If these lobes are due to the fusion of the primary buds, it is evident that the right bud has produced the greater part of the ventral pancreas. In the older specimen (fig. 6), there is no trace of a left lobe. But in other cases, as shown in figs. 5 and 7, from embryos of 6.0 mm. and 10 to 12 mm. respectively, both lobes are well defined; in both



Figs. 4 to 7 Models of the ventral pancreas in pig embryos. × 120 diam. Fig. 4, 5.1 mm. (H. E. C., Ser. 1409). Fig. 5, 6.0 mm. (H. E. C., Ser. 918). Figs. 6 and 7, '10 to 12' mm. *D. ch.*, ductus choledochus. *Duo.*, duodenum. *Int.*, intestine. *L. d.*, right lobe of the ventral pancreas. *L. s.*, left lobe of the ventral pancreas. *P. d.*, dorsal pancreas. *Pr. v.*, ventral process of the dorsal pancreas.

cases the left lobe is longer but more slender than the right. The four models just described explain the contradictory conclusions of Wlassow and Völker. Wlassow states that "the ventral pancreas is very distinctly two-lobed." Völker finds that "in its origin it is not two-lobed, since it bends at once to the right." Both forms were found by Hilton, who modelled the ventral pancreas in two embryos, the ages of which he estimated at 17½ and 20 days respectively. Presumably they measured between 6 and 7 mm. Since the bi-lobed form occurred in the younger specimen, Hilton suggested that it might be an earlier stage.

The student collection at the Harvard Medical School, which includes serial sections of 150 pig embryos measuring from 10 to 12 mm., affords an unusual opportunity for determining the frequency of the bi-lobed form. In seventeen specimens (11.3 per cent), the left lobe is well developed. In seven additional cases, a small wing or projection from the main mass suggests a rudimentary left lobe. When present, the left lobe varies in position. Usually it crosses the root of the ventral mesentery, and it may terminate near the dorsal pancreas (fig. 8). In three embryos, the left lobe descends along the mesenteric attachment, and in one of these it terminates within the mesentery (fig. 9). Frequently a nodule of cells, or a small cyst, is seen on the hepatic side of the mesentery (as shown at *x* in fig. 10). Because of the possibility that these may have been detached from the left lobe of the ventral pancreas, they were carefully examined.

Among 100 embryos, the nodules or cysts were found in 51 specimens. Often two or three occur in a single embryo. They are generally located close to the peritoneal epithelium and frequently they are found at the summit of a connective tissue elevation, as in fig. 10. This elevation does not always coincide with the line of mesenteric attachment. The nodules may occur at any point along the peritoneal covering of the gall bladder, and, near its tip, where the peritoneum invests it on all sides, they are found toward the ventral body wall. Occasionally they are seen deep within the connective tissue layer which surrounds the gall bladder, and often they are found in actual connection with the hepatic trabeculae at the sides of this layer. Some of them therefore arise from the trabeculae, and others are detached outgrowths of the cystic duct or gall bladder, with which two of them were seen to be connected.[1] After becoming detached, they may migrate along the peritoneum to the elevation where they are frequently found.

Although most of the nodules and cysts are of hepatic origin, it is still possible that some of them are derived from the pancreas. In two specimens, nodules indistinguishable in structure from that shown in fig. 10 were found on the intestinal side of the mesentery. It is quite improbable that these came from the liver. The largest cyst observed, which is shown in fig. 12, occurred in a 20 mm. embryo. It occupies the same position as the nodule in fig. 10. In structure, it closely resembles the mesenteric cyst found in another pig embryo of 20 mm., which was figured by Lewis and Thyng in vol. 7 of this Journal (p. 509). The mesenteric cyst was probably derived from an accessory pancreas, and it is possible that the cyst shown in fig. 12 is of pancreatic origin. That the left lobe of the ventral pancreas may extend across the ventral mesentery to the hepatic side, is shown in fig. 11, from an embryo of 6.0 mm. Moreover, a constriction suggests that the terminal part of this lobe may become detached. In an embryo of 8.0 mm., Wlassow found the left lobe extending still further across the mesentery, as seen in fig. 3 of his publication. Although corresponding conditions have apparently not been observed in the adult pig, it is of interest to note that in two cats, Heuer found lobes of the pancreas extending along the cystic and common bile ducts to the gall bladder.

At the Ithaca meeting of the American Association of Anatomists, Dresbach demonstrated a very interesting specimen of 'pancreatic bladder' in the adult cat. In such cases, which have been reported by Mayer, Gage, and Miller, the ductus choledochus is accompanied by another duct, which terminates in an expansion resembling the gall bladder, to which it is closely applied. Moreover its microscopic structure, as found by Dresbach, is

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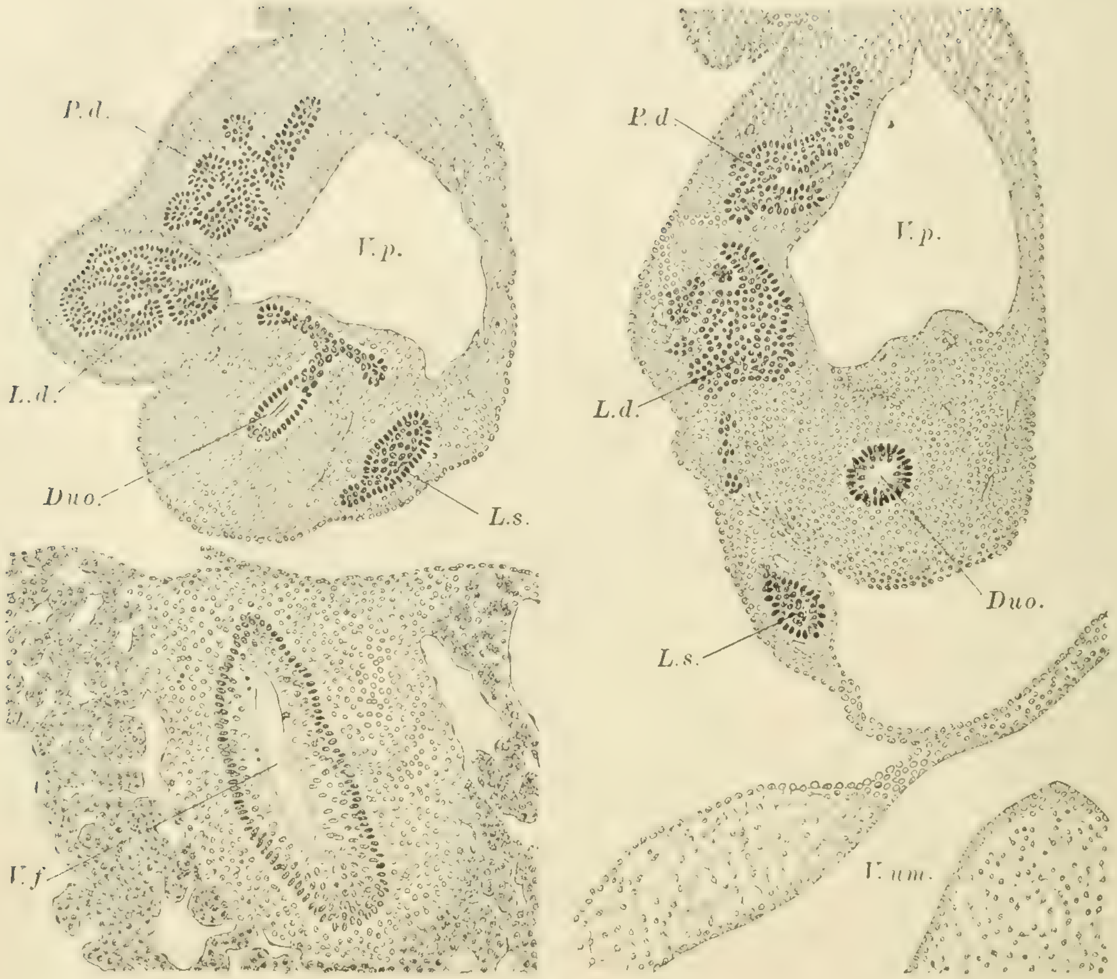
[1] In addition to the slender outgrowths referred to, the gall bladder presents rounded outpocketings, which are being studied in this laboratory by Dr. H. Bernstein.



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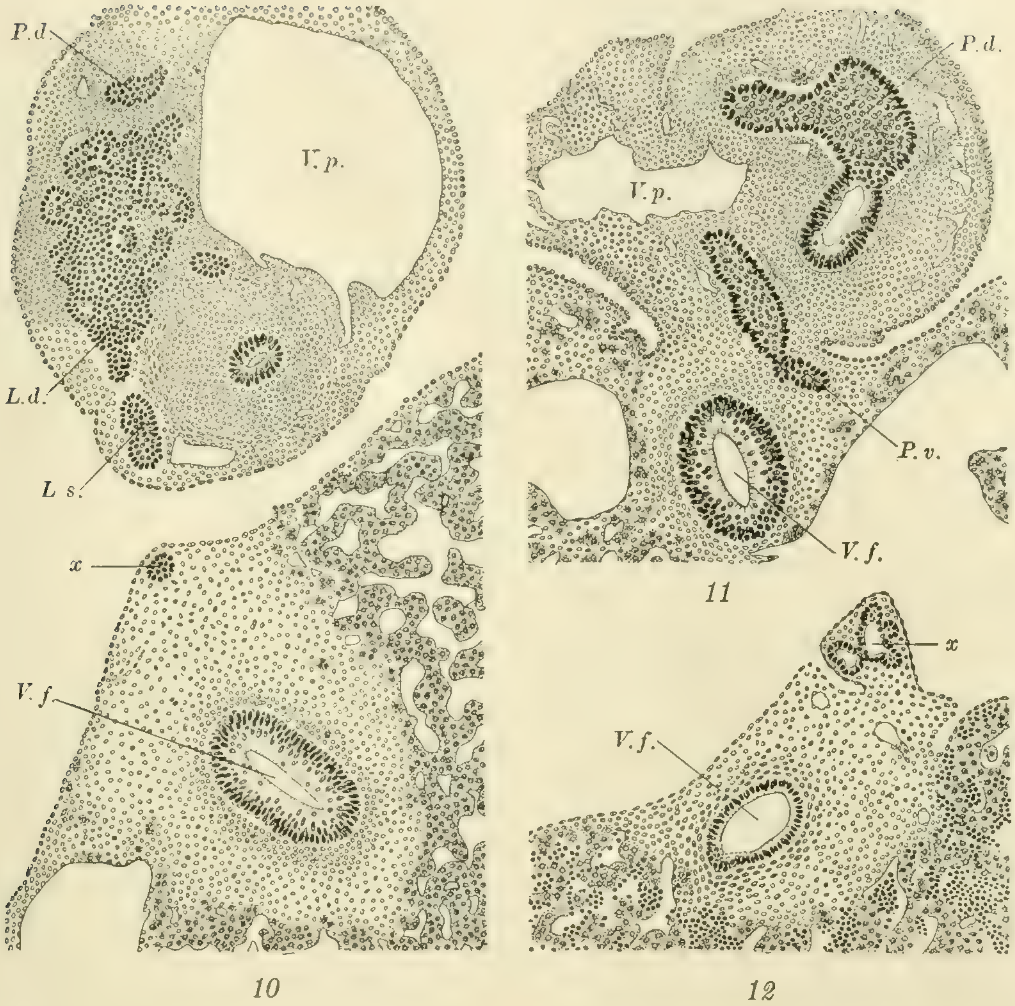
Figs. 8 and 9 Transverse sections of pig embryos of '10 to 12' mm. × 75 diam. *V. f.*, gall bladder. *V. p.*, portal vein. *V. um.*, umbilical vein. For other abbreviations, see figs 4 to 7.

"essentially like that of the gall bladder and its duct." Thus the cases suggest an extreme subdivision of the gall bladder and hepatic diverticulum; but Miller is doubtless correct in correlating them with embryonic conditions in the pancreas. He considers that they are degenerate left lobes of the ventral pancreas, terminating in cysts, and having little or no pancreatic tissue along their ducts.

When the left lobe tends to encircle the intestine, instead of extending into the ventral mesentery, it gives rise to a very different anomaly—the annular pancreas. It may fairly be said that the embryo shown in fig. 8 already possesses such a pancreas,



Figs. 10 to 12. Transverse sections of pig embryos. × 75 diam. Fig. 10, '10 to 12' mm. Fig. 11, 6.0 mm. (H. E. C., Ser. 1705, Sect. 252). Fig. 12. 20.0 mm. (H. E. C., Ser. 59, Sect. 977). *P. v.*, appendage of the left lobe of the ventral pancreas. *x*, epithelial nodule or cyst. For other abbreviations, see Figs. 4 to 7.

although at this stage the circuit of the intestine is not quite complete. Baldwin has recently reviewed the cases reported in man, to which he adds another. In explaining the anomaly, he assumes that "ordinarily the left half of the ventral pancreas atrophies," and concludes that the anomaly is due, either to a persistence of the left half, or to an excessive growth of the right half, which takes place ventral to the duodenum and extends to the left. He does not decide between these two possibilities. Since then, Miss Cords has reported another case. She does not refer to right and left halves, or lobes, but considers that in her case the entire ventral pancreas has grown around the duodenum to the left. Fortunately she has published excellent figures of the ducts, which show clearly the embryonic relations, since there is no anastomosis between the ducts of the dorsal and ventral pancreases. The duct of the ventral pancreas is seen to bifurcate. Its right branch, soon subdivided, corresponds to the normal right lobe. Its long left branch, which has led to the anomaly, corresponds closely to the left lobe which we have described in the pig embryo (figs. 7 and 8). It is perhaps more like one of the pig embryos not figured, in which the right lobe is rudimentary and the median stem appears continuous with the left lobe.

Lecco, who studied the annular pancreas in the adult and sought in human embryos for an explanation of the anomaly, justly criticizes Miss Cords's work as follows:

"A glance at the familiar figures in Keibel and Elze's *Normentafel*, as well as the examination of the embryos placed at my disposal, shows what a great distance separates the two pancreases, making a fusion of the two, ventral to the duodenum, seem highly improbable."

He adds that in Baldwin's paper he finds no evidence for either of the explanations proposed by Baldwin (cited above). The evidence, indeed, can be found only in abnormal embryos having annular pancreases in process of development. Baldwin apparently did not examine embryos, and Lecco studied only normal ones; but in certain of the pig embryos described above, the anomaly is almost complete, and it is due to a left lobe of the ventral pancreas.

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THE ACOUSTIC COMPLEX AND ITS RELATIONS IN THE BRAIN OF THE OPOSSUM (DIDELPHYS VIRGINIANA)

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From the Anatomical Laboratory of the University of Michigan

FOURTEEN FIGURES

The following study of the central acoustic complex has been undertaken by the writer with the primary object of producing by reconstruction methods a simple three-dimensional picture of this apparatus, which would while of specific application to the opossum, yet have a larger value in the development of a clear-cut conception of the morphology of this group of related structures in the mammalian brain as a type. The work of Sabin has established the value of this method of approach in the study of the anatomy of the brain; and it is hoped that this paper may prove a contribution to the field in which she has been so distinguished a pioneer. It may be added that this study is among the first of a series now in progress in this laboratory, whose eventual purpose is to present a morphological survey of the entire brain of Didelphys Virginiana.

As a form in which to study the acoustic complex, the opossum offers several decided advantages, chief among which from the standpoint of this paper is the clearness with which units and relations stand out in a relatively primitive mammalian brain, but little obscured by the massive pontine nuclei and their connections which characterize the higher forms. Differentiation by the method of Weigert being especially satisfactory for a comparatively gross study at low magnification, the models were made from such preparations. The drawings of individual sections serve the double purpose of elucidating the models, and of presenting certain important features whose incorporation in the reconstructions was deemed inadvisable.

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In carrying on the present study, the basic work of Held upon the central acoustic path has been constantly referred to; likewise the studies of Cajal and v. Kölliker and the compilation of results given in Barker's text. From the standpoint of reconstruction the work of Sabin upon the medulla of the new-born child has been applied wherever possible as a guide to the region under consideration. The reconstruction work of Streeter upon the cranial nerves and upon the embryology of the peripheral auditory apparatus, that of Essick upon the corpus ponto-bulbare, and the as yet unpublished work carried on in this laboratory by Calhoun upon the cranial nerves of the opossum other than the eighth, have been similarly employed.

As regards the investigation of the brain in Monotremes and Marsupials, the publications of G. Eliott Smith and the work of Ziehen, require mention. Their studies, however, did not extend to the fiber-paths of the hindbrain. Published work on the opossum brain itself has been confined apparently to a paper by Herrick on the olfactory apparatus, giving a brief description and a few typical sections of the region studied. The immediate field of this paper is therefore comparatively virgin. In fact the central acoustic apparatus considered alone, has never been the subject of three-dimensional reconstruction purely for its own sake—so that it is hoped that in this direction the present study will prove a serviceable aid in the visualization and comprehension of this important division of the central nervous system.

It may be stated at this point that the study of the seventh nerve that appears in the reconstructions, is incidental. Morphologically its relations to the eighth makes it a valuable landmark, and it is largely for its value in this direction that it was included in the modelling.

MATERIAL AND METHODS

The material upon which the study was carried out consists of three sets of serial sections, in the transverse, sagittal and horizontal planes respectively, of the entire brain of the adult opossum. These sections are the property of this laboratory and were prepared by Dr. R. R. Pinckard. The sections were cut at

50 microns and stained by the Weigert-Pal method. Two additional transverse series which were prepared by Dr. McCotter and Dr. Kollig, later became available for comparison.

In preparing the reconstructions the Born wax-plate technique has been followed with slight modification. The drawings were made with the projection lantern and corrected from the sections with the binocular microscope. The sheets were incorporated in millimeter plates, the selected structures being then cut out and piled. Models were made from sections in all three planes, and each used as a check upon the other. After making some preliminary models it was found that the best results on the whole were obtained from the transverse sections. Two finished models from this set were accordingly made, and it is from these that the illustrations for this paper are taken. Of the two models, one was made with the parts rigid, the restiform body, the pons and the brachium conjunctivum being included together with the seventh nerve as landmarks. The other is a dissectible model including nothing but the cochlear and vestibular complexes and the seventh nerve. It was found possible to so construct this model that the separate groups could be shown independently. From it the drawings of the two individual systems were made. Reconstruction was carried anteriorly only far enough to include most of the corpus geniculatum mediale. The course of the auditory path anterior to this point was found to be too indefinitely defined to permit of satisfactory rendition by this method. The relation of the superior nucleus of the vestibular to the floor of the cerebellum and its basal nuclei was also rather baffling. In such cases as the latter, where the models do not clearly show the points involved, resort has been made to drawings of the sections themselves, which it is hoped will aid the reader in following the text.

It has become increasingly evident to the writer as this study has progressed that the approaching of the problem from the standpoint of finer histological detail, as obtained by the Golgi and Cajal silver reduction methods, would be essential to the reaching of conclusive results on many of the points of anatomical structure mentioned in this paper. Although a Golgi series is now available in this laboratory, it was finally decided, however, that the scope of the present study might well be limited to the Weigert preparations.

Finally, it should be understood that the drawings of the models herewith presented represent an effort to reduce the apparatus under consideration to something of its simplest terms. Accordingly disputed points and minute details have been largely omitted, and attention called to such omissions in the text. The corpus ponto-bulbare, and the anterior commissural tract in relation with the nucleus lemniscus lateralis are so distinctive in position and relations that they are represented in spite of the fact that they may still be considered as disputed points.

COCHLEAR APPARATUS

In the general topography of the opossum hindbrain, the relatively enormous size and prominence of the primitive sensory and motor groups forming the central apparatus of the cranial nerves, is as striking as is the enormous development of the pons in the human brain. Such sensory groups as the fifth and the eighth stand out with startling distinctness, and the identity of the main units in the structure is seldom in doubt for a moment. Even on the external surface of the brain stem the bulge of the spinal fifth, and the corpus trapezoideum and its offshoot in the form of the lateral lemniscus, are easily made out. The posterior colliculus and the medial geniculate body are even more striking external landmarks. The opossum brain at first glance therefore, impresses one as especially well adapted to the mapping out of large primitive sensory groups by reconstruction.

The general structure of the cochlear apparatus is essentially the same in the opossum as in the more familiar types, and its main divisions may be similarly outlined, somewhat as follows. The chain of auditory conduction begins with the cochlear nerve proper, consisting of the axones of bipolar cells in the spiral ganglion. This cochlear nerve is accompanied by the vestibular nerve, the two together forming the eighth cranial or acoustic nerve of ordinary nomenclature. The cochlear nerve terminates in two nuclei of reception, the dorsal and ventral, the dorsal cochlear nucleus being identical with the so-called tuberculum acousticum. From these two nuclei of reception the auditory path is generally represented as continuing in part by way of the corpus trapezoideum on the ventral surface of the brain stem and in part by way of the striae acousticae on the dorsal, to the nuclear masses associated with the corpus trapezoideum, and to the lateral leminiscus and its associated ganglion mass. The distribution of these centrally directed fibers among the fiber tracts in question and their terminations in the superior olive and the nuclei of the corpus trapezoideum and the lateral lemniscus, are still among the unsettled questions in the finer structure of the central acoustic path. The proportion of acoustic fibers in the striae has also been shown to vary in different mammalian brains. It is now well recognized, however, and the point is of significance in the study of the models, that most of the fibers from the cochlear nuclei of one side of the brain cross the median line and are either interrupted in the superior olive or in the nucleus corporis trapezoidei, or are continued into the lateral lemniscus, of the opposite side. The corpus trapezoideum is therefore essentially a decussating path connecting the cochlear apparatus and more especially the cochlear nuclei of *one* side with the nuclear masses and the lateral lemniscus of the *other*. The value of keeping this relation in mind in studying the model is apparent. In the elongated grey mass of the nucleus of the lateral lemniscus a portion of the fibers which cross by way of the corpus trapezoideum or originate from cells in the nuclear masses of that tract, are interrupted. The fibers of the lateral lemniscus terminate in considerable numbers in the nuclear mass of the inferior colliculus. A portion of the fibers of the lateral lemniscus are prolonged beyond the inferior colliculus, and joined presumably by fibers from cells within the nucleus of the inferior colliculus, proceed by way of the brachium colliculi inferioris to the corpus geniculatum mediale, whose nucleus constitutes the last of the sharply defined stations in the path. From this point the remnants of the lateral lemniscus, and other groups of fibers associated with the eighth apparatus, are prolonged cerebralward.

Before proceeding to the detailed description of the models, certain respects in which they represent conditions peculiar to the opossum may first be pointed out.

The typical situation of the cochlear nuclei in such brains as those of the cat, rabbit and man, is largely external to the corpus restiforme, the tuberculum being lateral to and somewhat above the ventral nucleus, and largely overlying it. In the opossum this is not the case. The tuberculum acousticum lies entirely medial to the corpus restiforme, and all but the more anterior part of the ventral nucleus is similarly placed. The anterior portion of the ventral nucleus escapes from under the corpus restiforme as it ascends into the cerebellum, and extends downward and backward between the entering strands of the cochlear nerve. This arrangement of the nuclei of reception makes it inevitable that the posterior half of the fibers of the N. cochlearis should be obliged to pierce the lower part of the restiform body on their way to the cochlear nuclei. If the opossum brain be regarded as the more primitive type, it is not difficult to conceive that with the crowding of the primary structures in the medulla by the relative increase in size of the cerebellar peduncles, the inferior olive and the pons in higher types of brain, the nuclei of reception of the cochlear apparatus have been forced downward and outward along the root of the cochlear nerve and over the top of the corpus restiforme to their present superficial position.

Discussion of the relations of the dorsal cochlear nucleus calls attention to a second structure in the opossum brain that requires special mention in the description of the model. Essick in 1907 described for the human brain a grey mass on the lateral surface of the medulla in the region of the seventh and eighth nerves, of which he had been unable to find any account in the literature. This he designated the corpus ponto-bulbare, and called attention to its connection with the pons. In his histological description mention is made of certain fibers from it which join the striae acousticae. No other connection with the cochlear apparatus is noted. Streeter ('03) in the figures accompanying his description of the floor of the fourth ventricle labels this mass the nucleus tela chorioidea inferior, presumably from its close association with the choroid plexus of the fourth ventricle. It is mentioned but not described in the text. It has been repeatedly figured in atlases of the brain, but apparently without immediate connection with the tuberculum acousticum. It is easily seen in the gross in the brain of the pig embryo, appearing as a welt-like strip passing from beneath the ventral lip of the lateral recess, downward and forward around the lateral aspect of the corpus restiforme between the roots of the seventh and eighth nerves, forward to the caudal border of the pons at the level of entrance of the fifth nerve. It is continuous dorsally with the tissue from which the ganglion masses of the eighth nerve, and especially the tuberculum acousticum, originate. In the opossum, evidences of connection with the tuberculum acousticum are even more decided (figs. 2, 3 and 5). In the gross it appears as a prominent band beginning at the caudo-lateral border of the tuberculum acousticum and extending over the restiform body downward and forward over its lateral aspect, then almost horizontally forward, immediately above the roots of both the seventh and eighth nerves, to the pons, where it merges with a capsule of grey matter high up on its posterior border. Mention of its appearance in the sections will be made below. The homology between this structure and the one described by Essick may not at first sight appear entirely undoubted, owing to a discrepancy between the positions of the mass in relation to the seventh and eighth nerves in the opossum and in higher mammalian brains. The writer is of the opinion however, that such a difference is readily explained by displacements due to the greater development of the pons in the higher types, and by the difference in position of the cochlear nuclei in the latter as compared with the former.

A third distinctive feature of the cochlear apparatus in the opossum relates to the course of centrally directed fibers from the cochlear nuclei. The close relation of the corpus trapezoideum to the ventral cochlear nucleus is easily made out in the sections and shows quite plainly in the models. This constitutes the ventral conduction path, which however, is not confined exclusively to axones from the ventral cochlear nucleus. The dorsal conduction path in most animals is derived to a considerable extent in similar fashion from the tuberculum acousticum, and in the form of the striae acousticae, which form a variable portion of the so-called striae medullares in different mammalian brains, passes dorsally over the restiform body, across the floor of the ventricle towards the median line and downward in the raphe, or by a more direct route downward on the medial side of the tractus spinalis N. vestibularis, to be distributed to the superior olivary nucleus of the same side or to the corpus trapezoideum to undergo decussation to the opposite side. In the human brain it has been shown that the striae medullares have relatively little to do with the auditory conduction path, though in the cat and rabbit they are largely auditory. In the opossum the dorsal conduction path is a prominent feature, and as in other animals investigated, is apparently largely derived from the dorsal cochlear nucleus. That this is not the exclusive source, however, is readily seen from the numerous fibers having apparently a distinct origin in the mass of the ventral nucleus, which pass upward along the lateral aspect of the tractus spinalis N. vestibularis, and arching over it, join the dorsal path as it passes downward on the medial aspect of the vestibular tract. The opossum brain does not, however, present any definite group of striae medullares passing across the floor of the ventricle. The entire picture is usurped by the dorsal path, the variation in whose course as regards the corpus restiforme is due to the above described relation of that tract to the cochlear nuclei. The corpus restiforme being external to the cochlear nuclei, the fibers of the dorsal path simply collect on the medial border of the tractus spinalis N. vestibularis and without any deflection towards the median line, pass directly downward through the anterior portion of the medial vestibular nucleus to the superior olivary nucleus. While it should remain with histological study by Golgi methods to determine whether this tract represents the exclusive course of fibers of the dorsal path, it is obviously the principal one in the opossum. Since it is at best no more than a homologue of the striae acousticae it was designated in the models for convenience as the olivo-cochlear tract, a name rather more descriptive of its course than that of striae acousticae.

A fourth feature of the model of the cochlear apparatus requiring special mention is the incorporation in it of two groups of fibers not concerned in the main conduction path. The first of these is the so-called peduncle of the superior olive (Stiel der kleinen Olive), which has been repeatedly described, and represents a connection of the auditory path with the nucleus of the sixth nerve. The second is a group of fibers passing from the nucleus lemnisci lateralis towards the median line. This group is present unmistakably in the opossum, has been described especially by von Kölliker, ('96), and mentioned by Held ('93), Cajal ('09) and others, and figured for the cat and the new-born child. In spite of doubt as to their exact origin and distribution it was thought desirable to include them in the model.

Passing now to the detailed description of the cochlear apparatus as modelled, it is easy by reconstruction to distinguish in the root of the eighth nerve its two component parts which with the root of the seventh nerve are somewhat diagrammatically rendered in the figures. All three nerve roots lie at about the same level on the wall of the medulla in the opossum, posterior to the pons and the point of entrance of the fifth nerve. The most posterior division represents the N. cochlearis and appears as a robust fiber tract piercing the corpus trapezoideum at an upwardly directed angle, close to its posterior border. As already pointed out, the most anterior portion of the entering nerve passes entirely below the corpus restiforme as it ascends into the cerebellum. The ventral cochlear nucleus, which is first reached by the fibers, is an irregularly oval mass of cells, flattened dorso-ventrally, lying in part below and in part medial to the restiform body and prolonged laterally and posteriorly into the root of the cochlear nerve, in the form of masses of cells between the entering strands. The posterior portion of this lateral extension is cut off from the mass of the nucleus by the restiform body, but is continuous with the main ganglion mass anteriorly. Part of the fibers of the entering nerve pass directly through the ventral nucleus, still at an upwardly directed angle, to reach the tuberculum acousticum which lies above and medial to the ventral nucleus.

The tuberculum acousticum, or dorsal cochlear nucleus, presents the appearance of an elongated mass of cells uniting the extremities of two peduncle-like fiber tracts, the one lateral and posterior, the other medial and anterior; the former consisting of those entering fibers of the N. cochlearis which are distributed to the tuberculum acousticum, and the latter of the dense strands of fibers constituting the olivo-cochlear tract, which pass downward from the tuberculum acousticum towards the superior olive and the nucleus corporis trapezoidei. The anterior end of the dorsal cochlear nucleus is separated from the ventral nucleus, which at this point lies lateral to it and in the same horizontal plane, by a deep notch, completely filled by the fibers of the nucleo-cerebellar tract of the vestibular nerve, as they pass upward toward the cerebellum. At the point where the N. cochlearis enters the tuberculum, the nuclear mass spreads out in the shape of a mushroom, becoming continuous on the dorsal surface of the restiform body with the corpus ponto-bulbare, already described. The larger part of the ganglion mass of the tuberculum lies at the point of entrance of the cochlear fibers, just lateral to the spinal tract of the vestibular. The remainder of the nucleus forms a bridge over the spinal vestibular tract, merging into the olivo-cochlear tract on the medial side. The tuberculum thus "straddles" the spinal vestibular, so to speak, the nerve forming one leg and the olivo-cochlear tract the other. The long axes of the two cochlear nuclei present a characteristic inclination to each other, that of the ventral nucleus being horizontal and extending antero-posteriorly, that of the dorsal being oblique and extending forward, downward and inward.

Of the central connections of the two cochlear nuclei, the olivo-cochlear tract has been described above. The figures from the model, owing to the necessity of representing a group of strands as a solid tract, give a somewhat exaggerated idea of its calibre, which however, may be corrected from the sections. The corpus trapezoideum or ventral path, is readily seen as a broad strip on the ventral and ventro-lateral surfaces of the unsectioned opossum brain, lying completely exposed posterior to the pons, owing to the comparatively low development of the latter. It is separated from the pons on the lateral aspect of the medulla by an interval occupied by the entering fibers of the fifth nerve. The fibers of the corpus trapezoideum follow the curve of the surface of the medulla, slanting somewhat forward, fibers coursing parallel and the whole tract bulging somewhat immediately below the entrance of the eighth nerve as a result of lateral displacement by the spinal root of the fifth. The main mass of the fibers is anterior to the point of entrance of the cochlear nerve, and even in the gross can be seen to be principally associated with the ventral cochlear nucleus. The seventh and the N. vestibularis pass directly through this thickest portion of the corpus trapezoideum. On the ventral surface of the medulla, the origin of the lateral lemniscus appears as a distinct rounded off-shoot from the anterior margin of the corpus trapezoideum. It is easily identified in the unsectioned brain.

The internal surface of the corpus trapezoideum is shown by reconstruction to present a median rounded elevation extending across it from the posterior to the anterior border, where it becomes indistinguishable from the rounded internal surface of the lower portion of the nucleus lemnisci lateralis. This elevation corresponds to the nucleus corporis trapezoidei and especially in the more prominent posterior portion, to the nucleus olivaris superior. Into the summit of this mass are received the fibers of the olivo-cochlear tract. Attention should be called to the close relation between the nucleus of the superior olive and the nucleus of the seventh nerve, which lies immediately posterior to it, and in actual contact with it. Anteriorly, the bifurcation of the superior olivary nucleus with the formation of median and lateral lobes is better shown in the sections (fig. 7). From the more anterior portion of the superior olive springs also the olivary peduncle already mentioned.

The continuation of the cochlear conduction path into the mesencephalon by way of the lateral lemniscus appears in the opossum as a dense mass of fibers, springing from the anterior border of the corpus trapezoideum. This tract, which at its origin is about half as wide as the corpus trapezoideum, passes rather abruptly towards the roof of the Sylvian aqueduct, following the curve of the outer surface of the brain stem forward, laterally, upward and finally medially towards the nucleus of the inferior colliculus, whose base it enfolds "wei ein Kelch" (von Kölliker). The lemniscus is deeply grooved along its medial surface, the groove broadening and becoming shallower as it ascends. Within this groove covered by a thin layer of fibers lies the elongated cylindrical ganglion mass of the nucleus of the lateral lemniscus. As the fibers of the lemniscus diverge those along the dorsal margin, which constitute the larger proportion, pass almost directly upward toward the nucleus of the inferior colliculus. The more anterior and ventral portion of the tract apparently ascends rather more gradually in the direction of the superior colliculus and the thalamus, the exact destination of the fibers not being determinable from the sections. A portion of them may form a direct extension of the conduction path cerebralward, without interruption in the inferior colliculus. The nucleus of the lateral lemniscus at its lower end can hardly be said to be continuous with that of the superior olive. There is a distinct gap, covering a number of sections in the transverse cuts, between the point where the highly characteristic structure of the superior olivary nucleus entirely disappears, and the point where definite indications of a lateral lemniscus, with its sharply defined nucleus appear. This gap is filled with a reticulated mass containing some ganglion cells apparently, which if anything, partakes of the structural appearance of the nucleus corporis trapezoidei. There appears to be no difficulty in recognizing the anatomical identity of the nucleus lemnisci lateralis in the opossum at least.

The group of fibers extending inward and downward toward the median line from the upper portion of the nucleus lemnisci lateralis has already been mentioned. In the separate model of the cochlear apparatus (fig. 3), the same allowance should be made for its conspicuousness as in the case of the olivo-cochlear tract. It is less prominent in fig. 2, owing to its position along the upper posterior border of the brachium conjunctivum, from which, however, it appears to be fairly distinct.

The inferior colliculus of the opossum brain is a large and conspicuous mass of ganglion cells and fibers forming the wall and roof of the posterior portion of the Sylvian aqueduct. It is of a somewhat uncertainly defined egg shape, the long axis slanting downward and forward at an angle of about 45°. It is divisible into two parts, a medial and a lateral, the medial being the more distinctly nuclear mass and the lateral the more fibrous portion. The medial, nuclear division is roughly bean-shaped, tapering to an indefinite wedge-like lower extremity that becomes indistinguishable from the central grey substance. The lateral division contains the main bundles of fibers of the lateral lemniscus as they pass to their termination in the nucleus colliculi inferioris and to the decussation of the inferior colliculi. It also includes the fibers of the brachium colliculi inferioris and those fibers of the lateral lemniscus whose doubtful destination has already been mentioned. This lateral portion of the colliculus may be regarded anatomically as a capsule-like investment of the medial portion, roughly triangular in shape, with its apex at the middle geniculate body. The brachium colliculi inferioris, uncertainly defined within the colliculus itself, becomes a well-marked group of fiber bundles as it approaches the middle geniculate. The fibers decussating between the two inferior colliculi are gathered apparently from both nucleus and capsule. The decussation is in the roof, well forward in the colliculus.

The corpus geniculatum mediale so far as reconstructed, is an oval mass perhaps one-fourth or one-fifth as large as the nucleus of the inferior colliculus, which forms a distinct protuberance on the external surface of the brain stem, somewhat below the median horizontal plane. Like the nucleus colliculi inferioris it has a partial capsular investment of fibers along its upper and posterior borders, composed largely of strands from the brachium colliculi inferioris, which enters it on its posterior medial surface. Anteriorly and above are other investing strands, whose course and distribution could not be determined.

This completes the description of the general morphology of the auditory conduction path as reconstructed from the opossum brain. Certain points better brought out by the typical sections presented will be taken up after consideration of the vestibular reconstruction.

VESTIBULAR APPARATUS

A reasonably correct understanding of the structure of the central apparatus for the vestibular nerve is a matter of comparatively recent development, and its conduction relations are only beginning to be elaborated with any very great degree of detail. Especially is this true of the cerebellar relations of the vestibular complex, knowledge of which is still in a rather unsatisfactory state. Enough is definitely known, however, about the apparatus as a whole to serve as guide to its reconstruction. This has been done by Sabin ('01) for the human brain in her general reconstruction of the medulla and mesencephalon of the new-born child. As a guide to the present work the following brief résumé condensed from Barker's summary of the conclusions of von Bechterew, Flechsig, Baginski, von Monakow, Sala, Held, Cajal and others, and Sabin's description of her model, may be of service.

With the central vestibular apparatus there are now known to be associated at least four definite nuclei, (1) the medial (Schwalbe's), (2) the lateral (Deiter's), (3) the superior (Bechterew's) and (4) the nucleus of the spinal root. In relation with these are three large groups of fibers, the ascending and descending roots, and the nucleo-cerebellar tract, the latter containing fibers from various parts of the system, passing to the basal nuclei of the cerebellum. The entering fibers of the vestibular nerve bifurcate at once on gaining the interior of the medulla, the descending branches passing downward in the descending limb or spinal root, many of them (according to Cajal, most of them) terminating in this immediate region. All their terminations are not fully known, however. The ascending limb fibers pass upward in the medulla in the ascending root, some of them to terminate in the superior and lateral vestibular nuclei, others to ascend into the cerebellum to terminate in connection with the nuclei of the roof. Of the nuclei, the medial and lateral and the nucleus of the spinal root are quite well defined and easily recognized. The exact limits of the superior nucleus are more doubtful, at least in the opossum. The medial nucleus lies in the floor of the fourth ventricle adjacent to the median line, and in close association posteriorly with the nucleus N. hypoglossi, and below with the nuclei of the N. vagus and the N. accessorius. Anteriorly its relation to the cochlear nuclei varies with the position of these in different brains. The cells of the nucleus of the spinal root as the name indicates, lie among the fibers of the spinal root itself. This brings its position immediately lateral to the medial nucleus, the adjoining borders of the two not being sharply differentiated from each other. The lateral nucleus (Deiter's) apparently consists of two parts as described by Sabin for the human brain, one medial and the other lateral to the spinal root of the vestibular nerve, and both approximately opposite, the point of entrance of the nerve. The division is only apparent however, since typical Deiter's cells can be recognized among the strands of the spinal tract, uniting the two divisions of the nucleus. The superior nucleus of the vestibular nerve lies in the line of continuation anteriorly of the vestibular spinal and ascending roots, in close association with Deiter's, and so wedged between it and the nucleo-cerebellar tract in higher mammals that it was at first regarded as an appendage of that nucleus. Whatever may be said for its definiteness in brains like the human, in the opossum it is outlined with difficulty. The ascending tract is distributed in part to the superior nucleus, as already mentioned, and in part to the basal nuclei of the cerebellum, especially the nucleus fastigii.

Decussation between the vestibular complexes of the two sides of the brain occurs between the fibers of the nucleo-cerebellar tract by way of the decussation of the two fastigial nuclei, and anteriorly between fibers from the two superior nuclei, opposite the point of origin of the brachium conjunctivum.

The nucleus cerebello-acousticus is the name under which Cajal has called attention to the existence of a group of cells in the lateral wall of the ventricle, in the margin of the ascending limb of the spinal tract as its cerebellar fibers ascend towards the ventricular roof, which receive collaterals from the ascending root. Such a structure cannot be definitely determined in Weigert preparations of the opossum hindbrain.

Three other groups of vestibular connections are usually described, namely, the internal arcuate fibers from the medial and spinal nuclei, a bundle from Deiter's nucleus to the spinal cord, and two groups of cerebellar connections, the medial and lateral bundles. These groups are not capable of satisfactory reconstruction in the opossum, and in fact distinct cerebellar groups could not be definitely identified.

In general the vestibular complex of the opossum corresponds quite closely to the general conception outlined above. Several points, however, require special notice.

From time to time in the literature relating to the nuclei of the floor of the fourth ventricle, stress has been laid upon the separate anatomical identity of the nucleus intercalatus, and its relation to the medial vestibular nucleus. A considerable number of authorities regard the former as part of the latter. In a recent paper ('03), already referred to, on the floor of the fourth ventricle, Streeter in endeavoring to correlate external and internal topography, discusses the status of the nucleus intercalatus. After reviewing the opinions since Staderini and van Gehuchten, he directs attention to the fact that Weigert preparations of the human brain give no ground for differentiating the two. Other methods of investigation however, show the question to be less easily settled. He advances as evidence for giving the nucleus intercalatus an identity of its own, among other things the existence of a neuroglia partition between it and the medial vestibular nucleus, demonstrable by special neuroglia stains, and also certain evidence from a brain in which one half of the cerebellum was congenitally absent, in which degenerative changes tending to establish the separateness of the two nuclei could be demonstrated. This same question came up at the outset in the reconstruction of the medial vestibular nucleus in the opossum. The use of Weigert preparations may of course lay the work open to the objection pointed out by Streeter, and the writer makes no pretence of urging the finality of his conclusions on this point from such evidence. None the less, he is obliged to confess that the preparations upon which this work has been done offer not the slightest excuse for differentiating the nucleus intercalatus from the nucleus medialis N. vestibularis. From the median line to the border of the spinal tract, the uniformity of the structure and staining reaction are absolutely unbroken, there is not the slightest sign of a partition, the felt-work of fibers is characteristic throughout, and even a significant dividing groove in the ventricular floor is apparently lacking. If the nucleus intercalatus is to be distinguished from the medial vestibular nucleus, it becomes impossible to reconstruct the latter in the opossum from Weigert preparations. The writer therefore followed what seems a legitimate conclusion from this material, and included all the area labelled as such in fig. 10, in the medial nucleus.

A second feature of the opossum brain that has to be reckoned with in the reconstruction of the anterior portion of the vestibular complex, is the imperfect differentiation of the basal structures of the cerebellum, especially the nuclei; and the confusing intimacy of the vestibular relations with these nuclei. The opossum cerebellum has no dentate nucleus, a fact which probably has its influence upon the primitive confusion of the base, since the functions and connections thus differentiated fall presumably at least in rudiment to the basal nuclei. The nucleus fastigii is a rather indefinitely defined structure, the chief clue to whose identity is its relation to the superior peduncle anteriorly. One other nuclear mass, medial to the nucleus fastigii and below, can be recognized in some of the sections. Figs. 6, 7 and 8 may serve to convey some idea of the difficulties of reconstructing in this region. The decussation in the roof seems to offer an exception to this rule, its parts standing out with considerable clearness.

A third point requiring brief mention in the same connection relates to the intimacy of the association between the superior nucleus of the vestibular nerve and the sensory nucleus of the fifth nerve, below it. The commissural connection of Bechterew's nucleus seems to be associated with a decussation between the two fifth nuclei, rather than with that of the superior peduncle as described by Sabin ('01) for the human brain. In the cross sections, boundaries for Bechterew's nuclei cannot be assigned, but the horizontal sections are somewhat more satisfactory in this respect.

Finally, in studying the figures of the models, the following points should be remembered: (1) certain connections of a minor nature or requiring special consideration are dealt with in discussing the typical sections shown; (2) all of the vestibular above the level of Deiter's may be regarded as in the cerebellum, not the medulla; (3) the view of the apparatus from above (fig. 4) represents it with a piece cut from the nucleo-cerebellar tract in order to give a better conception of the position and relation of Deiter's nucleus, and the connection between its two apparently separate parts.

Passing now to the detailed description of the vestibular system as reconstructed for the opossum, the N. vestibularis appears as a fiber bundle of somewhat smaller size than the N. cochlearis, the root being composed of a number of separate fasciculi, easily demonstrated in careful examination of the gross specimen, in contrast to the single solid bundle of fibers presented by the cochlear root before it reaches the lateral surface of the medulla. The vestibular root enters just anterior to the cochlear, at a slightly sharper angle, some of its strands underlying those of the latter nerve. The restiform body passes up into the cerebellum so far posterior as to lie entirely above the level of entrance of the vestibular nerve, and so forms no part of the picture in this immediate area. As soon as the root has pierced the corpus trapezoideum, bifurcation into ascending and descending roots occurs. This bifurcation takes place in a region rather closely confined by surrounding structures. Laterally the larger part of the root and its divisions is overlaid by the anterior part of the ventral cochlear nucleus. The root of the seventh nerve is closely applied to the anterior and lower border of the vestibular, the ascending root of the latter passing into the superior nucleus and the cerebellum above the anterior limb of the genu. Just medial to the point of bifurcation the olivo-cochlear tract passes downward through the medial vestibular nucleus to the superior olive. In the same way the cerebellar part of the ascending tract is surrounded medially, posteriorly and postero-laterally by the cochlear nuclei and on its antero-lateral aspect bears a shallow depression in which lies the corpus restiforme. Just as the dorsal cochlear nucleus may be said to "straddle" the spinal vestibular, so the spinal vestibular in the region of origin of its two divisions may be said to be squeezed between the two peduncle-like fiber tracts associated with the cochlear reception nuclei. No sooner is the pressure relieved than both the vestibular fiber-tracts expand considerably, the anterior to its distribution in the cerebellar nuclei and the superior vestibular nucleus, and the posterior to accommodate the cells of the spinal nucleus.

Of the nuclear masses, the medial possesses perhaps the most characteristic shape. It lies as previously remarked in the floor of the fourth ventricle, its lateral border fused with the spinal tract. The triangular area, apex posterior, which corresponds to it, just lateral to the elevation marking the spinal tract is easily recognized in the floor of the ventricle in the unsectioned brain. The form of the dorsal surface and the longitudinal groove in the ventral surface made by the sensory fifth, together with the deep notch medial to it for the nucleus N. vagi and the tractus solitarius are well shown in figs. 2 and 4. Anteriorly the medial nucleus is pierced by the olivo-cochlear tract in the manner described above. The slender tongue extending horizontally forward along the medial side of the olivo-cochlear tract toward the region of the superior nucleus, will be noted again in connection with the sections.

The nucleus of the spinal tract of the vestibular nerve takes its form from that of the tract among whose fasciculi it lies. The combined mass is therefore roughly a long cone, tapering towards a blunt point posteriorly, but represented as cut across to allow for as yet undetermined caudal extension. The cross section posterior to the tuberculum acousticum is round or an oval slightly flattened dorso-ventrally. In the region of greatest constriction, the flattening of the dorso-medial and dorso-lateral surfaces and the greater compactness of the mass is gained largely at the expense of the grey matter, which is relatively small in amount here, though most abundant just posterior to this region. The boundaries of the medial and spinal nuclei and the spinal tract are not as distinct in the model as in the sections. Their form, however, can be made out in figs. 5, 10 and 12.

The ascending tract of the spinal vestibular runs a comparatively short course from the bifurcation, beginning almost at once to break up into its component parts for distribution. Some strands continue horizontally forward into the substance of the superior nucleus. Another portion breaks up in Deiter's nucleus. A large number of strands, however, turn abruptly upward in a vertical direction, forming a flattened column in a depression in whose lateral surface lies the upper and outer part of Deiter's nucleus. The posterior and medial fibers of this column fill the notch separating the anterior extensions of the two cochlear nuclei. On reaching the level of the nucleus fastigii in the base of the cerebellum, these fibers, in company with others forming the nucleo-cerebellar tract, bend sharply medially and spread out like a fan into the nuclear masses of the base of the cerebellum, a well-defined portion participating in a decussation with fibers from the opposite side. This decussation is very easily distinguished from that of the restiform body, the complete separation of the two being very beautifully shown in horizontal sections through the base of the cerebellum. The restiform body appears as a dense compact mass, lying lateral to the ascending nucleo-cerebellar tract, and passing upward at an inclination which brings its point of decussation well anterior to that of the vestibular group. The discrete smaller bundles and ribbon-like strands constituting the latter are totally different in appearance from the compact solidity of the former.

The distribution of fibers of the ascending path to the superior nucleus follows the same general arrangement of ribbon-like strands, curving around towards the medial side in the anterior end of the superior nucleus, in a manner which serves to some extent as an index to the boundary of the nucleus in this direction.

The outlines of the superior vestibular nucleus as previously noted, are not very definite. The mass lies in the lateral wall of the ventricle and forms in a general way a blunt prolongation of the long axis of the descending root. It is much cut up by strands of fibers ascending towards the cerebellum or terminating in its substance. Its close relation to the nucleus of the fifth nerve, especially the sensory division, which lies immediately below it, corresponds to that noted in the general description. Externally it is of course entirely concealed from view by the middle and posterior cerebellar peduncles and the overhanging mass of the great flocculus-like lateral extension of the opossum cerebellum itself. Internally the point of origin of the commissure from the superior vestibular nucleus, in association with fibers from the sensory nucleus of the fifth, is suggested in fig. 4. The slender anterior projection of the medial nucleus, which is also suggested, and its connection will be referred to in discussing the sections.

The lateral nucleus of the vestibular nerve as contrasted with the superior, is easily recognized and outlined, owing chiefly to the highly characteristic large ganglion cells of which it is composed. It lies opposite and above the point of entry of the nerve and corresponds in its apparent division into two parts, to the outline description already given. The continuity of the two can be readily made out, however, in the horizontal sections especially. The upper part of Deiter's therefore appears as a bulge in the side of the nucleo-cerebellar tract while the central part lies within the ascending limb of the spinal tract itself, and the lower forms a tongue or wedge-like piece inserted between the olivo-cochlear tract and the descending vestibular root.

DISCUSSION OF SECTIONS

(FIGS. 5–14)

The closing part of this paper is devoted to a discussion of the sections selected as illustrative of the topography of the two divisions of the central acoustic complex in the opossum, as previously discussed. While the sections have also a general interest from the standpoint of other groups of structures in the medulla, the intention in the present case is to confine the discussion to salient points connected with the eighth and incidentally the seventh nerves, and to direct attention to their bearing on the models and to particulars in which they fill out deficiencies in the plastic work.

The drawings constituting the figures under consideration were made by the use of the projection lantern, every possible detail obtainable being filled in in this way. The pen and ink work was then done on the projected outline, additional detail being obtained by the use of the binocular microscope, and every effort being made to make both detail and general effect correspond as closely to the original as the medium in use would permit. The diagrammatic has been studiously avoided, especially in connection with doubtful points. A system of abbreviated labelling was adopted, which it is hoped will be intelligible practically on first inspection, without the use of a key. Where conventional labelling appeared unsatisfactory, letters were used and referred to in the legend.

Inasmuch as the models were developed primarily from the transverse sections, consideration of the topographical detail may appropriately begin with them.

Fig. 5. Series A, slide 98, row 2, section 2. This section is taken at the level of the posterior part of the radix N. cochlearis (see figs. 1 and 2). It presents very satisfactorily the manner in which the fibers of the cochlear root pierce the corpus restiforme, and the medial situation of both cochlear nuclei as regards that tract. Indications of the imbricated character of the strands in the ventral nucleus, a very characteristic appearance in the region of entrance of the nerve in all the sections, and of the extension of the grey matter downward between the strands of the nerve, are apparent. The corpus ponto-bulbare and its connection with the tuberculum are prominent in this region. The connection with the tuberculum consists of a distinct band of fine fibrils arching over the dorsal surface of the corpus restiforme. In these preparations no signs of ganglion cells associated with them, either in the tuberculum or in the corpus ponto-bulbare could be made out. It may be noted incidentally that one of the newer series mentioned under the heading of Materials and Methods, owing to a more intense Weigert staining, exhibits this connection between the tuberculum and the corpus ponto-bulbare in a more striking fashion than does the section figured. As regards the vestibular, the close relation between the descending root and its

nucleus, and the medial nucleus is apparent, and the surface markings in the floor of the ventricle outlining their position can be inferred. Internal arcuate fibers are abundant in all sections involving this part of the vestibular complex, strands of them emerging from between the fasciculi of the descending root and passing toward the median line, or more directly downward among the bundles of the formatio reticularis. An interesting group of fibers is indicated by the letter *A*. This group appears in a series of eight or ten sections of which the most anterior ones begin to show the fibers of the corpus trapezoideum, from which however, it appears to be entirely distinct. The apparent origin is among the fasciculi of the descending vestibular root, and its termination in the nucleus of the seventh nerve, at whose lateral border the fibers scatter. Such scattering of course may be due to the interposition of an obstacle, and may not indicate an interruption in the path. I have not been able to find in von Kölliker's or Cajal's descriptions or figures any reference to such a bundle among the connections of the seventh nerve. It is of course possible that this represents a collateral of the vestibular system passing by way of the medial lemniscus to other levels in the mesencephalon or the cord. This section also shows the position and bilobed form of the seventh nerve nucleus, and the characteristic arrangement of the fine strands passing upward toward the genu. Throughout this and other sections more or less characteristic differences in size of ganglion cells may be made out without special staining. Those of the medial and spinal vestibular nuclei for example are quite small, those of Deiter's nucleus or the nucleus of the seventh nerve very large. The two latter are so conspicuous as to make the nuclei in question recognizable at first glance.

Fig. 6. Series A, slide 99, row 2, section 1. This and the following section are through the most complicated part of the opossum medulla, and present especially interesting pictures. This section is taken through the middle of the radix N. vestibularis and the genu of the facial nerve. The anterior part of the ventral cochlear nucleus is still prominent, but the tuberculum is represented only by a bit of grey matter from the anterior end. The

lower portion of the olivo-cochlear tract is conspicuous, and the contribution of the ventral nucleus to the dorsal path by way of this bundle is well shown. The principal origin of the tract in the tuberculum is more posterior, but is shown quite as satisfactorily in sagittal section (fig. 13). The corpus trapezoideum appears from origin to decussation. The origin in the substance of the ventral nucleus and the button-holing with the entering strands of the vestibular root are well shown. The lateral half of the bilobed superior olive and the termination of the ventral end of the olivo-cochlear tract in connection with it, can be better made out in the sections themselves, owing to the impossibility of doing justice in pen and ink work to the whorls and coils of delicate fibrils which give the nucleus a characteristic appearance. The posterior end of the medial half is just suggested and with the nucleus corporis trapezoidei appears to better advantage in the next section. The division of the decussation into a dorsal and a ventral part, the dorsal apparently concerned largely with fibers from the olives, and the ventral a more direct long-path decussation, can be well made out. The fibers of the sixth nerve should not be confused with olivary connections in this section. As regards the vestibular, the distribution of the main mass of fibers direct to the region of the spinal tract, along its ventral surface is apparent. Deiter's nucleus, with its large ganglion cells, easily the largest in this part of the brain, looms up, a conspicuous landmark in the field, above and medial to the division of the entering strands. The medial part is easily made out in this section. The most posterior strands of the nucleo-cerebellar tract of the vestibular nerve are also seen, and the dense strands cut at an angle which represent the more anterior fasciculi of the same tract as they bend back towards the more posterior parts of the nucleus fastigii. The restiform body in its groove on the outer side of the nucleo-cerebellar tract, stands out clearly. On the medial side in the roof of the ventricle, the characteristic appearance and relations of the vestibular decussation, especially its independence of the corpus restiforme, are apparent. Of the medial vestibular nucleus, nothing but the anterior tongue-like mass of fibers and grey matter lateral to the olivo-cochlear tract remains. This group of fibers can be better traced in horizontal sections. The region in the angle between this and the spinal vestibular tract is a matted mass of fibers which cannot well be represented in low power pen and ink work. Such a complex well deserves Golgi analysis. A portion of these fibers are undoubtedly a part of the connections of Deiter's with the vestibular tracts. Another part can be shown to be aberrant strands of the seventh nerve, which appears in the next few sections. A considerable number of them probably represent internal arcuates, and the connections of Deiter's with the median longitudinal fasciculus. Still others are fibers to the olivo-cochlear tract. An appearance perhaps striking enough to deserve mention, is found in the other half of this section. One coarse strand of fibers presents every appearance of being an olive-cerebellar connection, lying somewhat lateral to the remnants of the main band of olivo-cochlear fibers, and joining the nucleo-cerebellar path of the vestibular above. On going back and forth through this region repeatedly comparing sides, the writer has inclined to the conclusion, however, that this is merely an aberrant strand of the olivo-cochlear system, whose close relation to an ascending strand of the vestibular spinal tract produces the deceptive appearance.

Fig. 7. Series A, slide 88, row 2, section 2. This section is somewhat anterior to the previous one, and involves the radix N. facialis. The features of special interest center around the corpus trapezoideum and the olive and its connections.

The region marked *A* in this section represents the principal feature for discussion. In reviewing the descriptions of the corpus trapezoideum given by Cajal, von Kölliker, Edinger and Held, the writer has been unable to find any reference to a possible connection of the corpus trapezoideum and the ventral cochlear nucleus, with the cerebellar nuclei. In fact the absence of connection of the cochlear apparatus with the cerebellum seems to be emphatically pointed out by a number of writers as a main distinction between vestibular and cochlear systems. With this weight of opinion against it, the writer calls attention to this and other sections through this portion of the opossum brain with considerable diffidence, and with the full realization that Weigert preparations are no final settlement of the question, and that their most convincing appearances may be deceptive. The grey matter between the strands of the corpus trapezoideum in sections somewhat posterior represents to all appearances the extreme anterior limit of the ventral cochlear nucleus, traceable back to the main mass without a break. Those familiar with Cajal's figures will recognize in fig. 359 on page 819 of the Histologie du Systeme Nerveux, Tome 1 (Azoulay), on the right hand side of the section, approximately the same region, as he pictures it for the cat. Comparison of the figures here given (figs. 6 and 7) with Cajal's is very instructive as regards the differences between the opossum and higher mammalian brains in this region. The cerebello-acoustic or nucleo-cerebellar path is the only group of fibers in this region labelled in his figure. In the opossum, another group is apparent, consisting of fine fibrils from the grey matter of the before-mentioned anterior part of the ventral cochlear nucleus, which apparently pass upward on the lateral side of the vestibular nucleo-cerebellar tract, gathering into slender strands which closely skirt the medial border of the inferior brachium, but are always perfectly distinct from it. These fibers follow the general direction of the ascending vestibular fibers although apparently distinct from them also and turn medianward to be lost among the fibers of the roof. Somewhat more anteriorly these slender stands are replaced by the coarser ones shown in fig. 7—absolutely identical in appearance with the perfectly characteristic root bundles of the corpus trapezoideum, sharply defined, standing out clearly against a paler background, and in one section appearing as an almost continuous strip of fibers from above the level of the highest part of Deiter's to the middle of the dense mass of the undoubted corpus trapezoideum. The section drawn is only averagely good in showing this relation. It however brings out the entire difference in direction of the fibers from those of the spinal tract, and their striking individuality as regards origin at least. In the face of these appearances, which are confirmed by the newer series of transverse sections prepared in this laboratory since this work was begun, and of the apparent impossibility of confusing the fiber-group in question with any other structure described for this region, the writer feels obliged to contend that so far as such preparations will support a contention, this represents some form of connection, at least of the corpus trapezoideum and probably of the anterior part of the ventral cochlear nucleus, with the cerebellum.

The remaining features of the section concern the superior olive and the nucleus corporis trapezoidei. The two are easily differentiated and the bilobed structure of the former made out. The differentiation of the parts of the nucleus corporis trapezoidei is not very satisfactory. Of the three groups of fibers passing dorso-ventrally through the formatio reticularis, the one nearest the raphe is the sixth nerve, and the middle one of the three can be followed through several sections as the peduncle of the superior olive, connecting it with the nucleus of the sixth.

Fig. 8. Series A, slide 85, row 1, section 1. This section is through the sensory and motor roots of the fifth nerve, and presents the anterior limits of the vestibular apparatus and the decussation of fibers from Bechterew's nuclei, in association with a similar set of fibers from the fifth. As regards the cochlear apparatus, the anterior part of the corpus trapezoideum can be seen, and the beginning differentiation of the lateral lemniscus and the nucleus of the lateral lemniscus. Primarily as regards the topography of the region as a whole, the section serves to indicate the indefiniteness of boundaries between the fifth, the superior vestibular and the fastigial nuclei in the wall of the ventricle. The group of fine fibers marked *A* appearing in cross section are apparently continuations of the fibers of the anterior portion of the medial vestibular nucleus, which have already been referred to.

Fig. 9. Series A, slide 77, row 1, section 1. This section is taken through the posterior median portion of the inferior colliculi, just short of the decussation between the two. The fact that in the same section so much of the lateral lemniscus and its distribution, and the nucleus of the colliculus can be shown, illustrates the abrupt upward sweep of the tract. The capsule of lemniscus fibers on the lateral aspect of the nucleus is also well

shown. The section also pictures very satisfactorily the groove in the medial surface, in which lies the nucleus with its covering of scattered strands and explains its failure to show in the figure of the lateral aspect (fig. 1). The letter *B* directs attention to the group of fibers from the nucleus lemnisci lateralis towards the median line, which was mentioned above among the details included in the model of the cochlear apparatus. The fibers can be traced quite definitely to the median line and a part of them at least show every indication of connection with the median longitudinal fasciculus. In the sections where this structure is most conspicuous, decussation of most of its fibers with those of the opposite side undoubtedly occurs. Attention may also be called to a somewhat similar but smaller and shorter group of fibers from the region of the lateral lemniscus itself, marked *A* in the figure. They are lost in the central grey and show no signs of any relation to those of the opposite side. In Weigert preparations at least, they appear to be quite distinct from the group just described.

As regards the association of the first-mentioned group of fibers with any definite group of cells in the nucleus lemnisci lateralis the sections somewhat anterior to the one shown, present every indication that such is the case. In one of these sections a definite almond-shaped mass of cells, probably the anterior superior end or superior division of the nucleus lemnisci lateralis appears as the point of origin of the fine fibrils uniting to form the strands in question. Of the relation of the tract to the brachium conjunctivum little can be said from these preparations except that the fibers under discussion appear coincidently in the section with the first signs of an approaching decussation of the brachium, but are readily distinguished from these latter by their greater delicacy and the relative absence of clumping into strands. Before the decussation of the brachium has reached decided proportions the tract in question has completely disappeared from the field. The general impression gained from these preparations is that any relation to the brachium conjunctivum is incidental rather than fundamental.

Passing now to the horizontal sections, we find them rather better adapted than the transverse to the picturing of the general relations and structure of the vestibular system in the medulla.

Fig. 10. Series B, slide 75, section 1. This section is taken at the level of the broadest part of the combined medial and spinal nuclei of the vestibular nerve, and presents the arrangement of the structures in the right wall and floor of the fourth ventricle. The picture of the medial vestibular is one of the best in the series. In the general topography the brachium conjunctivum, the restiform body and the pons are conspicuous landmarks. The two cochlear nuclei appear in typical relation to each other and to the corpus restiforme, the anterior part of the ventral nucleus being also clearly shown in its association with the region of Deiter's and the nucleo-cerebellar tract. The corpus ponto-bulbare on the lateral aspect of the corpus restiforme is better shown in the following section. In drawing the medial and spinal vestibular nuclei an attempt was made to convey the effect of uniformity in staining reaction, which is so striking a feature of this mass, even in the best differentiated sections. The absence of any visible division into nucleus intercalatus and medial vestibular nucleus is apparent, the peculiar brownish background and the felt-work of exceedingly delicate fibers and small ganglion cells being uniform throughout. There is however, a marginal concentration of fibers along the inner border of the medial nucleus. Some of these pass posteriorly as a slender tail-like extension towards the anterior end of the hypoglossal nucleus. The larger part, however, appear better in the following figure, as the anterior tongue or extension already mentioned as lying on the medial side of the olivo-cochlear tract. The caudal indentation in the medial nucleus due to the nucleus N. vagi and the tractus solitarius, and the position of the fifth on the postero-medial border, are well shown. Of Deiter's only the lateral portion can be seen, among the strands of the nucleo-cerebellar tract. The typical inward curve of those strands of the spinal tract which pass into the nucleus superior N. vestibularis (the tract itself lies ventral to this section) gives an index to the anterior limits of the

nucleus, while the close association of the nucleus itself with the structures of the cerebellar base can be inferred from the proximity of the fibers of the superior brachium, originating in the nucleus fastigii.

Of the special features of this section, the one marked *A* is taken up with the next figure. *B* is also better shown in that connection. Particular attention is however, directed to *C*. This band of fine parallel fibers is a conspicuous object in five sections in this region, and can be made out in two or three more. In two or three of the best sections it can be traced definitely into the substance of the ventral cochlear nucleus, separating into fine strands in the nuclear mass. Ultimate origins or terminations could not be determined. The fibers follow the curve shown in the figure cerebralward scattering fan-like among the bundles and grey matter of the superior vestibular nucleus and the nucleo-cerebellar tract. The close association of this group of fibers with other more doubtful ones apparently forming cerebralward connections of the vestibular makes interpretation of them difficult, and in any case Golgi analysis would appear essential. In these preparations the group, however, presents every appearance of a cochlear-vestibular connection possibly uniting the ventral nucleus of the former with the superior of the latter. While a connection of the ventral cochlear nucleus with the cerebellum or midbrain by this route might be considered among the possibilities, such connection is hardly apparent in these sections. In the opinion of the writer, this fiber group corresponds to the one marked *B* in fig. 6, and should not be confused with the possible cochlear-cerebellar group mentioned under fig. 7, page 426, which lies lateral to it. See also fig. 6, where the latter group lies close against the medial surface of the restiform body.

Fig. 11. Series B, slide 79, section 2. This section is ventral to the one shown in the preceding figure, and with the following one is given primarily for the sake of certain special features of the vestibular system. The two parts of Deiter's nucleus are well shown, the large ganglion cells being inserted somewhat schematically in black. The anatomical connection of the two parts by scattered cells among the strands of the spinal

tract of the vestibular is also apparent. These cells are conspicuous in the sections owing to the brownish color they take from the mordanting and staining, so that the nucleus is usually easily defined. Under *A* in all three horizontal sections attention is directed to a prominent nuclear mass in the central grey substance, to which a majority of the fibers of the anterior extension of the nucleus medialis N. vestibularis, apparently lead. The region does not differentiate entirely satisfactorily in these Weigert preparations, and the fibrils are very delicate, which tend to make conclusions about them rather vague. The connection of the band of fibers with the medial vestibular nucleus is the more apparent of the two terminations of the group. The writer has not encountered any description of such a structure as this path or such connection with the central grey substance as it apparently presents, in any account of the vestibular system that he has seen. The nuclear mass in the neighborhood of the point of entrance of the fibers presents a considerable clump of fairly large ganglion cells. More medially the mass presents somewhat the appearance of the substance of the medial vestibular nucleus, the cells in this portion being very much smaller. The relation to the mesencephalic fifth is seen in fig. 10.

Fig. 12. Series B, slide 82, section 2. This section is taken still more ventral to the two preceding. The spinal tract and Deiter's are again conspicuous and the olivo-cochlear tract is seen in cross section as in the preceding figure. The chief feature of interest is the decussation between fibers from Bechterew's nuclei (marked *A*) in connection with a decussation of fibers from the fifth (marked *B*), posterior to it in the main, though in the transverse sections (fig. 8) the two are seen in the field at the same time. In the human brain, according to Sabin, this Bechterew decussation is as closely associated with the brachium conjunctivum as it is with the fifth in the opossum. This section also illustrates very satisfactorily the connection of the corpus ponto-bulbare with the posterior border of the pons. The presence of large numbers of delicate fibrils can only be indicated in the drawing. No ganglion cells can be distinguished in these preparations.

Finally we pass to a short consideration of the sagittal sections shown, which serve the purpose of presenting in panoramic style many of the features of the foregoing discussion.

Fig. 13. Series C, slide 80, section 2. This section is taken approximately midway between the raphe and the side of the brain stem. It is too far medial to show much of the cochlear nuclei, but presents in especially satisfactory manner the antero-posterior relations of the seventh nerve nucleus, the nucleus of the superior olive and the origin of the lateral lemniscus and its nucleus. The nucleus colliculi inferioris is also especially well shown, together with its decussation in cross section, which has not appeared in the preceding figures. The antero-posterior dimension of the vestibular system in the medulla is also given, including the piercing of the medial nucleus by the strands of the olivo-cochlear tract. The decussation of fibers from the superior nucleus of the vestibular in association with those from the fifth is seen in cross section anterior to the radix N. facialis. Some idea is also given of the sagittal section of the cerebellar base.

Fig. 14. Series C, slide 93, section 1. This section is taken lateral to the preceding one and is intended primarily to present the mesencephalic portion of the cochlear apparatus. The arrangement of the fibers of the lateral lemniscus, the nucleus lemnisci lateralis, the brachium colliculi inferioris and the medial geniculate body are ideally presented. Posteriorly the tuberculum acousticum is prominent and a good cross section of the corpus trapezoideum forms the ventral boundary of this part of the medulla. The close association of the anterior part of the vestibular complex and the sensory nucleus of the fifth is again apparent. The ascent of the nucleo-cerebellar tract to its distribution and descussation in the base of the cerebellum is not as well shown as in some of the preceding figures.

SUMMARY

1. The cochlear reception nuclei lie internal to the restiform body.

2. The corpus ponto-bulbare apparently is structurally connected with both the tuberculum acousticum and the pons. Its failure to follow the course between the seventh and eighth nerves described for other brains can be explained by the low development of the pons in the opossum, and the position of the cochlear nuclei, as compared with other mammalian brains.

3. No distinct striae medullares were recognized, the dorsal path of impulses from the cochlear nuclei being by way of an olivo-cochlear bundle to the region of the superior olive of the same side. This bundle receives fibers from both nuclei, but principally from the dorsal.

4. The ventral path is by way of the corpus trapezoideum, which originates largely in the ventral cochlear nucleus.

5. The corpus trapezoideum presents indications in these preparations of a connection with the base of the cerebellum anteriorly.

6. The anterior part of the ventral cochlear nucleus presents evidence of a connection with the base of the cerebellum in the region of the before-mentioned trapezoidal connection.

7. The ventral cochlear nucleus apparently presents a connection with the region of the superior nucleus of the vestibular system.

8. The superior olive in the opossum is distinctly bilobed, and is not anatomically continuous with the nucleus lemnisci lateralis.

9. The peduncle of the superior olive and the fibers passing to the median line from the superior end of the nucleus of the lateral lemniscus are easily recognizable in the opossum.

10. Of the vestibular system the superior, the lateral, and the medial nuclei, and the nucleus of the descending root, together with the bifurcation of the entering fibers into ascending and descending paths, and the nucleo-cerebellar tract, can be identified.

11. The comparatively undifferentiated cerebellar base makes the cerebellar relations of the vestibular apparatus uncertain.

12. The superior nucleus is poorly defined and differentiated with difficulty from surrounding structures, namely the nuclei of the base of the cerebellum above, and the sensory nucleus of the fifth nerve below and anteriorly.

13. The decussation between fibers from the two Bechterew's nuclei occurs in association with a decussation of fibers apparently from the sensory nucleus of the fifth nerve, rather than with the brachium conjunctivum.

14. The nucleus lateralis N. vestibularis (Deiter's) presents a medial and a lateral portion, separated by strands of the vestibular spinal tract, but characteristic Deiter's cells are found among the strands, showing the two parts to be essentially one nucleus.

15. So far as these preparations indicate, there is no nucleus intercalatus separate and distinct from the nucleus medialis N. vestibularis.

16. A band of fine fibers passing cerebralward from the medial vestibular nucleus along the floor and wall of the ventricle, medial to the olivo-cochlear tract, apparently connects this nucleus with a ganglion mass in the central grey substance in the floor of the ventricle anterior and medial to the superior vestibular nucleus.

17. A connection apparently exists between the spinal vestibular nucleus and the seventh nerve nucleus.

18. The strands of the nucleo-cerebellar tract are distributed to the large nuclear mass in the base of the cerebellum that apparently corresponds to the nucleus fastigii of the human brain. Decussation of these fibers takes place in the roof of the ventricle, and is easily distinguished from the decussation of the corpus restiforme.

The writer desires that the concluding paragraph of this paper shall be an expression of his sense of obligation to Professor Streeter, at whose suggestion this work was undertaken, and who with generous coöperation has made his own and the laboratory's resources freely accessible for its advancement.

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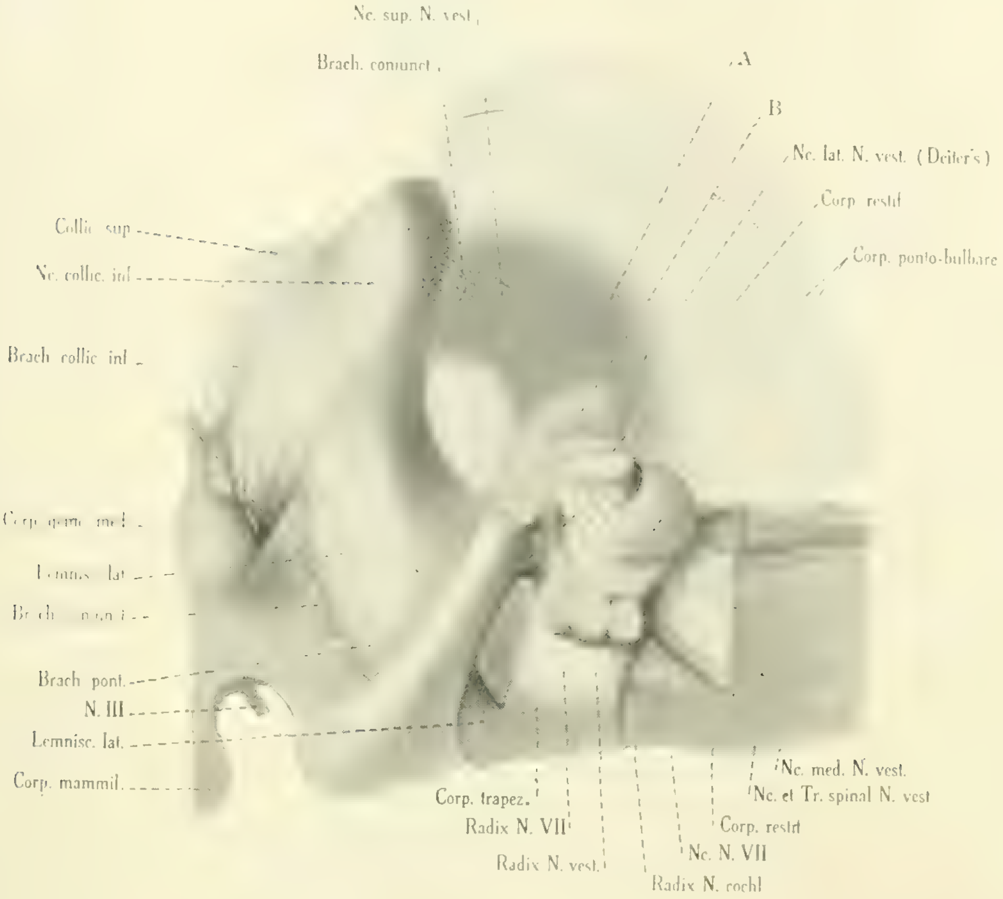
Fig. 1 Left lateral aspect of a wax-plate reconstruction of the cochlear and vestibular systems of the opossum brain. Enlarged 5 diameters. The corpus restiforme, the pons, the brachium conjunctivum and the seventh nerve are included as landmarks. *A*, decussating vestibular fibers from the nucleus fastigii and the nucleo-cerebellar tract; *B*, nucleo-cerebellar tract.

Fig. 2 Posterior aspect of reconstruction shown in fig. 1. *A*, cut surface of the nucleus fastigii. *B*, fibers passing from the superior end of the nucleus lemnisci lateralis to the median line, showing close relation with the decussation of the brachium conjunctivum; *C*, nucleo-cerebellar tract of the vestibular system and decussating strands.


Nc. sup. N. vest.
Brach. conjunct.
A
B
Nc. lat. N. vest. (Deiter's)
Corp. restif
Corp. ponto-bulbare
Collic. sup.
Nc. collic. inf.
Brach. collic. inf.
Corp. genic. med.
Lemnisc. lat.
Brach. conjunct.
Brach. pont.
N. III
Lemnisc. lat.
Corp. mammil.
Corp. trapez.
Radix N. VII
Radix N. vest.
Radix N. cochl.
Nc. med. N. vest.
Nc. et Tr. spinal N. vest.
Corp. restif
Nc. N. VII

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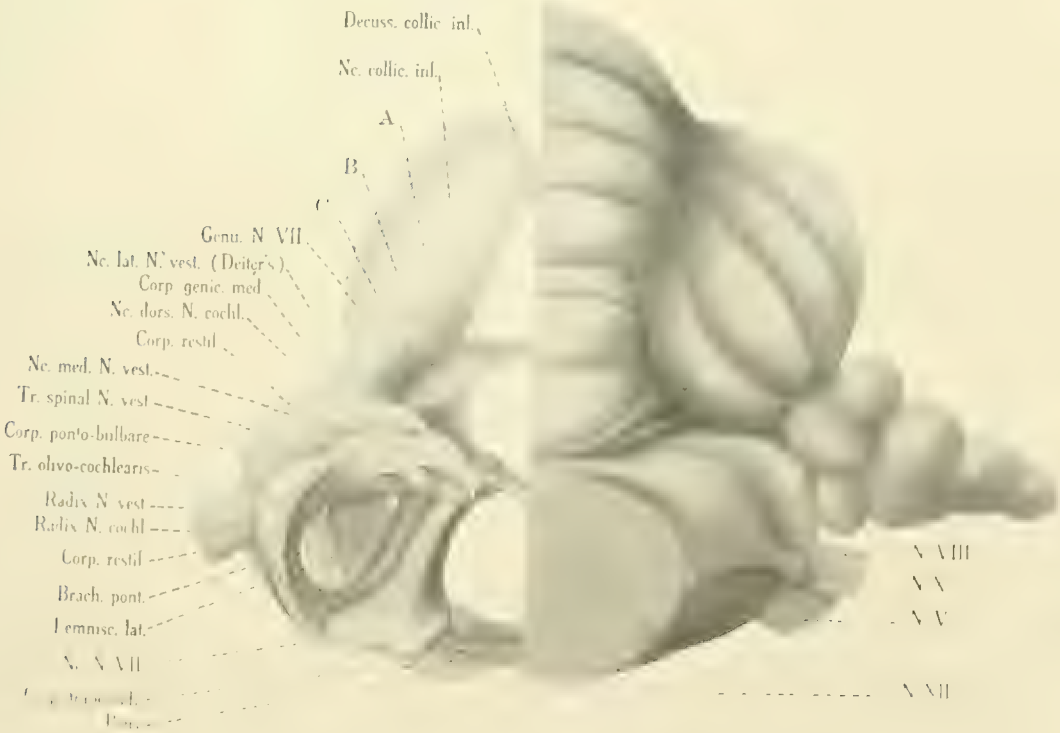
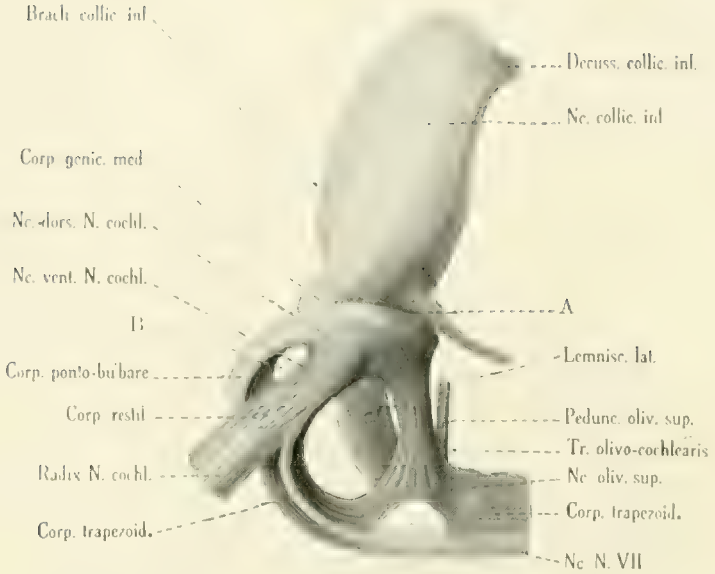

Decuss. collic. inf.
Nc. collic. inf.
A
B
C
Genu. N. VII
Nc. lat. N. vest. (Deiter's)
Corp. genic. med.
Nc. dors. N. cochl.
Corp. restif
Nc. med. N. vest.
Tr. spinal N. vest.
Corp. ponto-bulbare
Tr. olivo-cochlearis
Radix N. vest.
Radix N. cochl.
Corp. restif
Brach. pont.
Lemnisc. lat.
Nc. N. VII
N. VIII
N. X
N. V
N. VII

2



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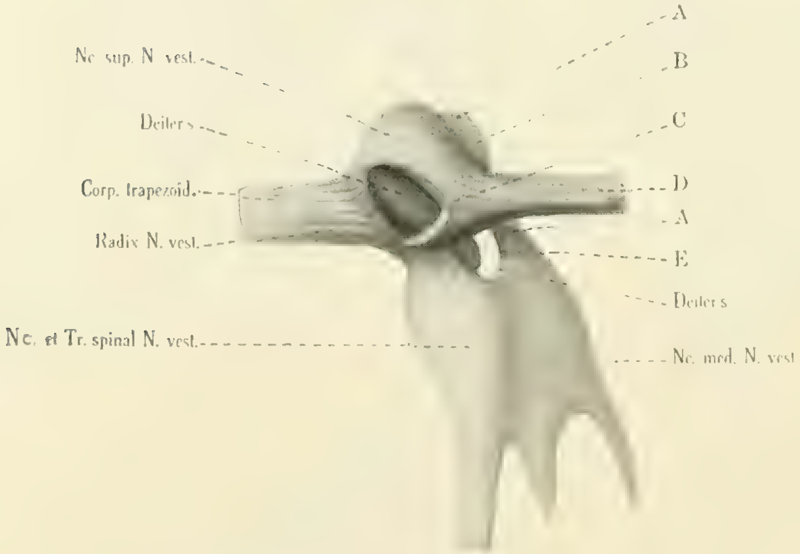

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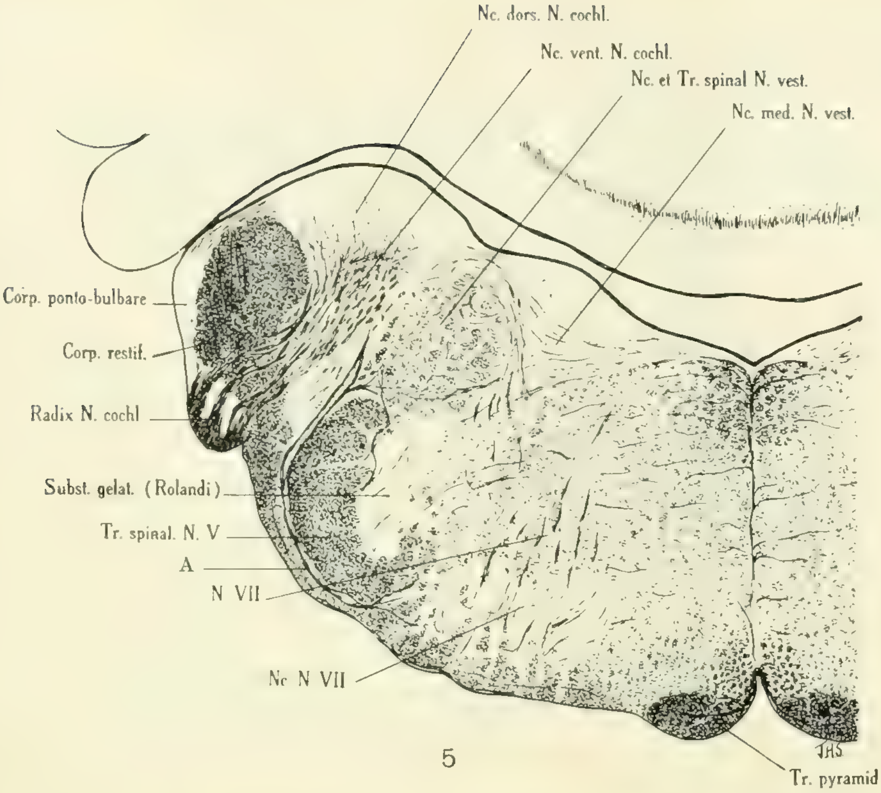
Fig. 3 Posterior aspect of a wax-plate reconstruction of the cochlear apparatus of the opossum brain. Enlarged 5 diameters. *A*, fibers passing from the superior end of the nucleus lemnisci lateralis to the median line (see *B* in fig. 2); *B*, opening through which passes the main mass of the corpus restiforme; *Corp. restif.* indicates the region in which the cochlear root is pierced by strands of the corpus restiforme.

Fig. 4 Dorsal aspect of a wax-plate reconstruction of the left vestibular complex of the opossum brain. Enlarged 5 diameters. *AA*, fibers passing from the medial nucleus cerebralward towards a nuclear mass in the central grey substance; *B*, fibers forming a decussation with similar ones from the superior vestibular nucleus of the opposite side; *C*, cut surface where a piece of the nucleo-cerebellar tract has been removed to expose Deiter's nucleus; *D*, decussation of vestibular fibers from the nucleo-cerebellar tract and the nucleus fastigii; *E*, opening through which pass the fibers of the olivo-cochlear tract or dorsal path of the cochlear system.



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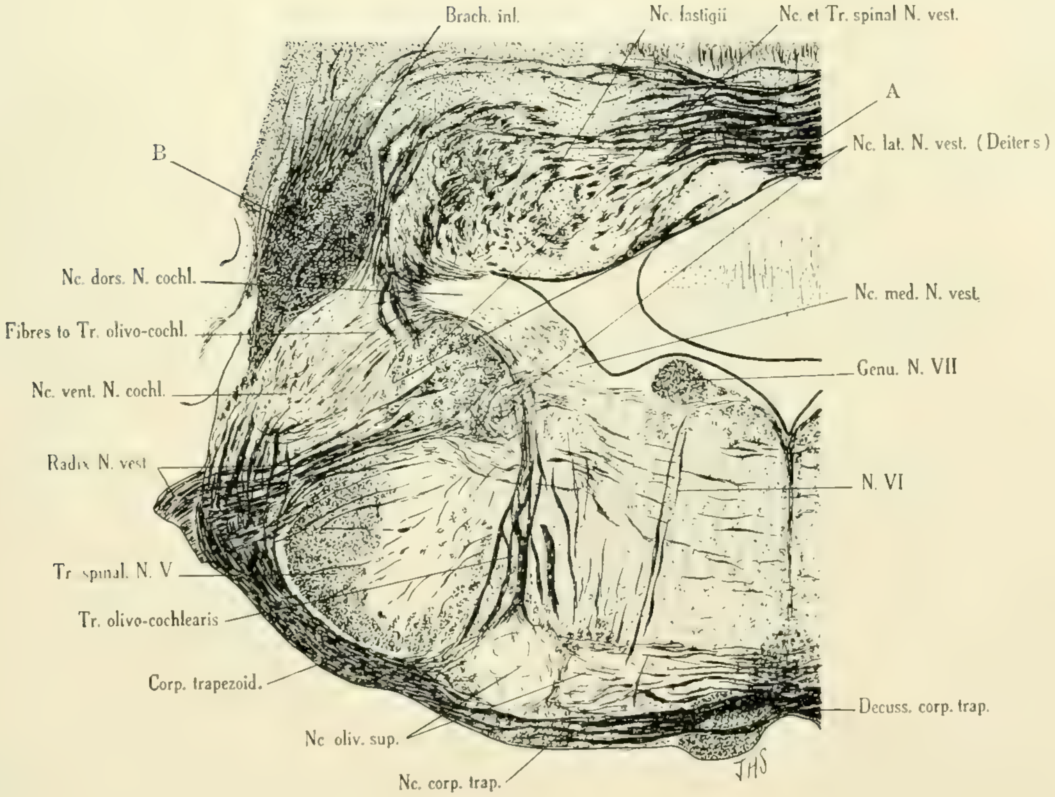
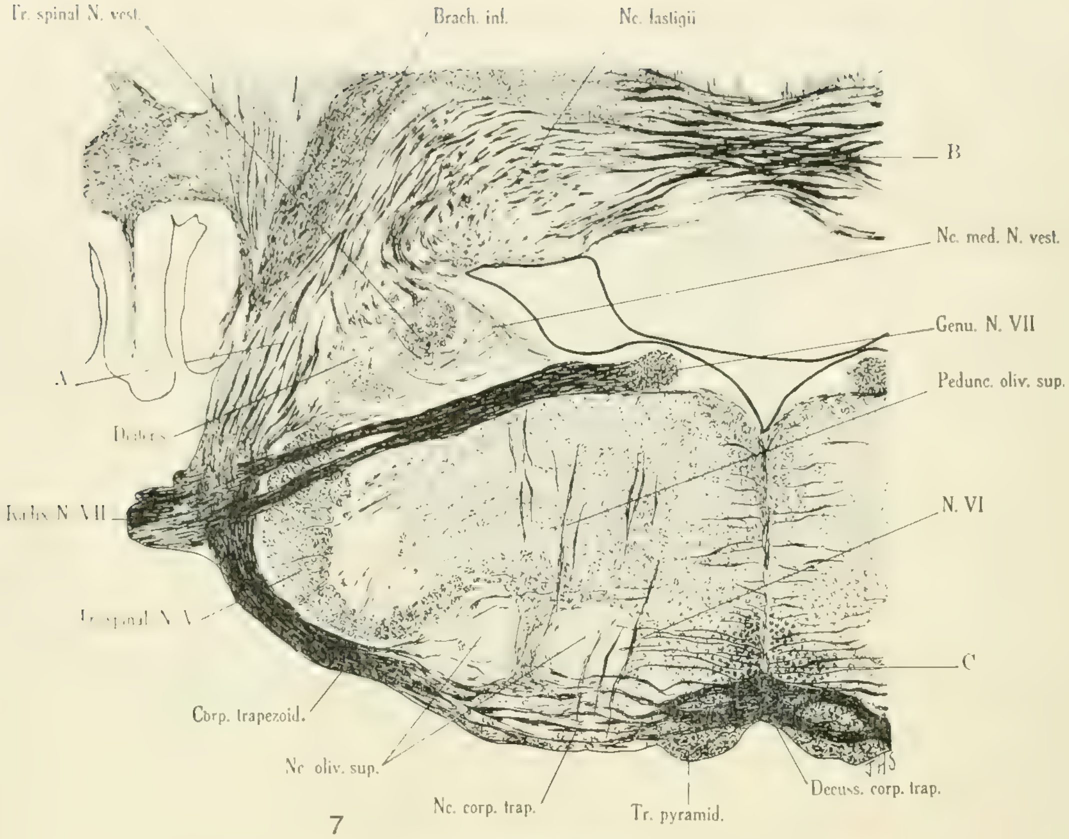


Fig. 5 Transverse section at the level of the Radix N. Cochlearis. Series A, slide 98, row 2, section 2. Enlarged 10 diameters. *A*, possible connection of N. vestibularis with N. facialis. For description of section, see page 422.

Fig. 6 Transverse section at the level of the Radix N. vestibularis. Series A, slide 90, row 2, section 1. Enlarged 10 diameters. *A*, vestibular decussation in the base of the cerebellum; *B*, cochlear-vestibular connection. For description of section, see page 423.



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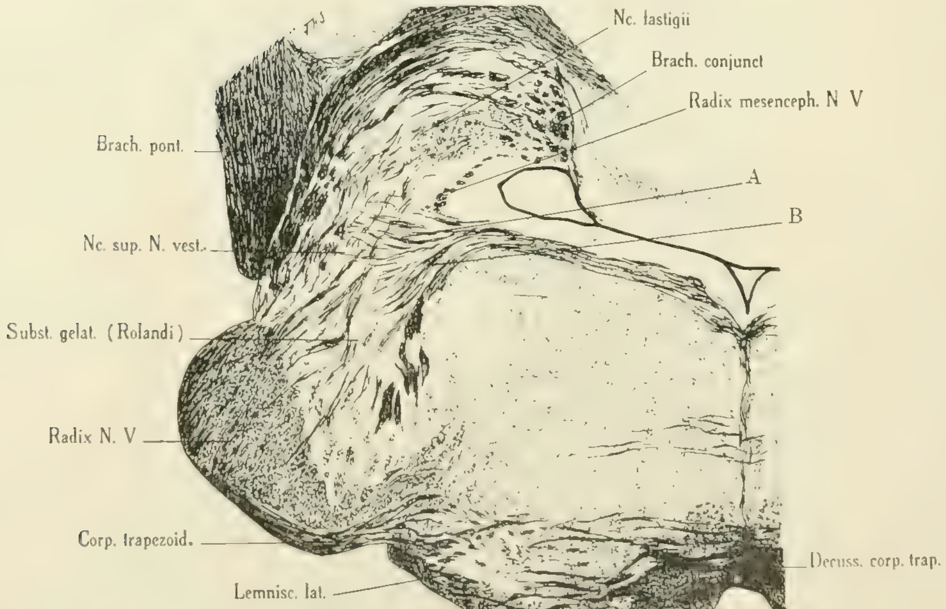

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Fig. 7 Transverse section at the level of the N. facialis. Series A, slide 88, row 2, section 2. Enlarged 10 diameters. *A*, possible cerebellar connection of corpus trapezoideum; *B*, vestibular decussation in the base of the cerebellum; *C*, possible inter-olivary portion of the decussation of the Corpus trapezoideum. For description of section, see page 425.

Fig. 8 Transverse section at the level of the radix N. trigemini and the superior vestibular nucleus. Series A, slide 85, row 1, section 1. Enlarged 10 diameters. *A*, decussating fibers from Bechterew's nucleus; *B*, decussating fibers from the nucleus N. trigemini. For description of this section, see page 427.

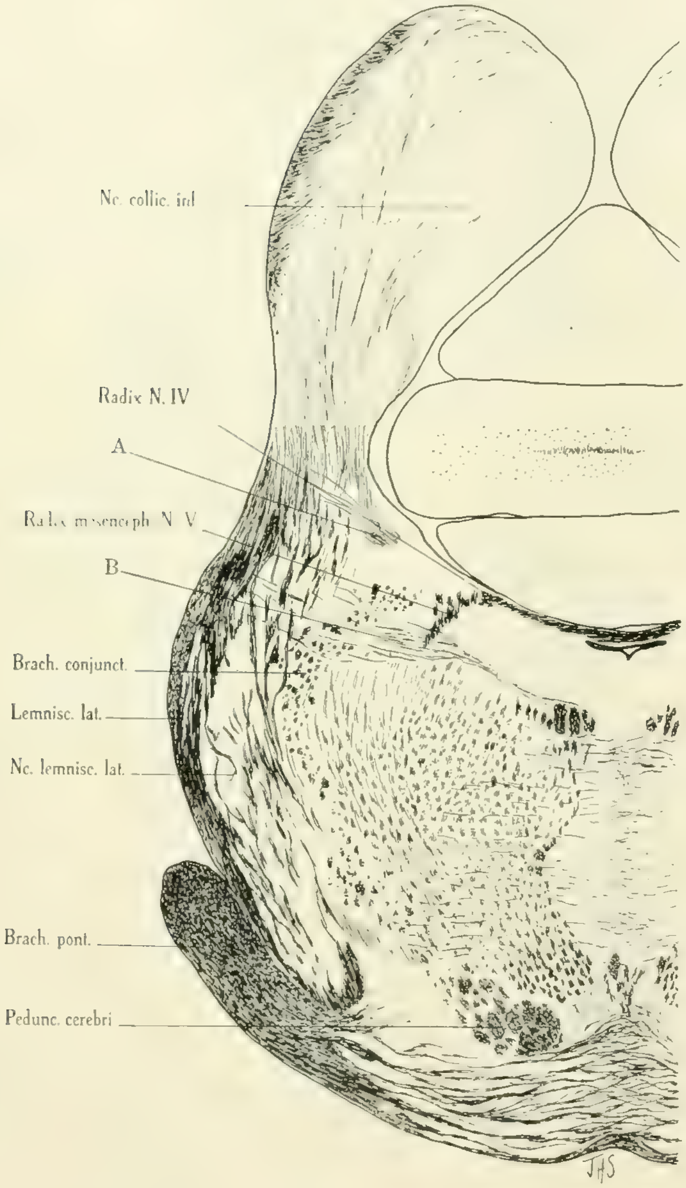


Fig. 9 Transverse section through the posterior part of the inferior colliculus. Series A, slide 77, row 1, section 1. Enlarged 10 diameters. *A*, see page 428; *B*, fibers from nucleus lemnisci lateralis to median line. For description of section, see page 427.

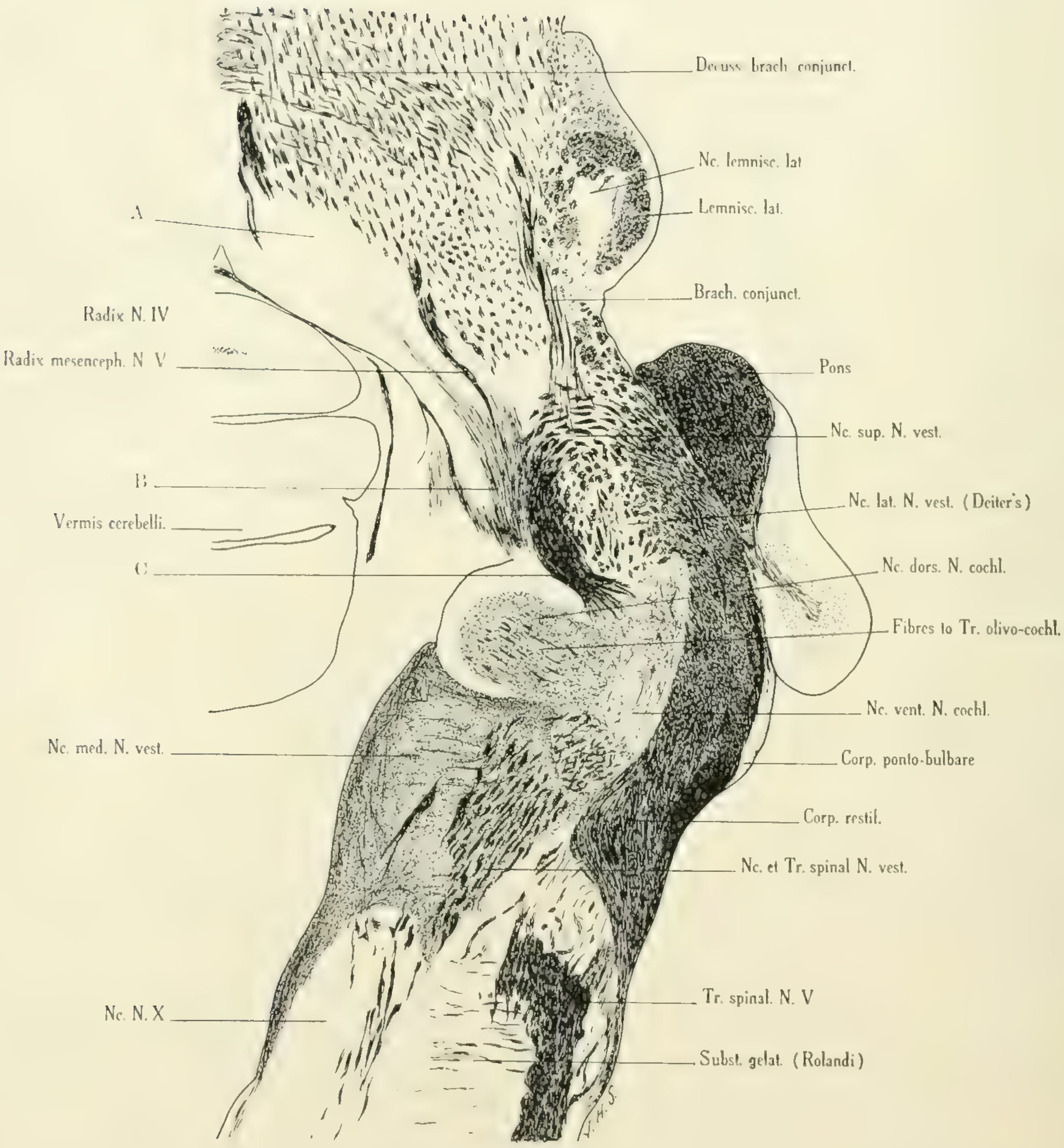
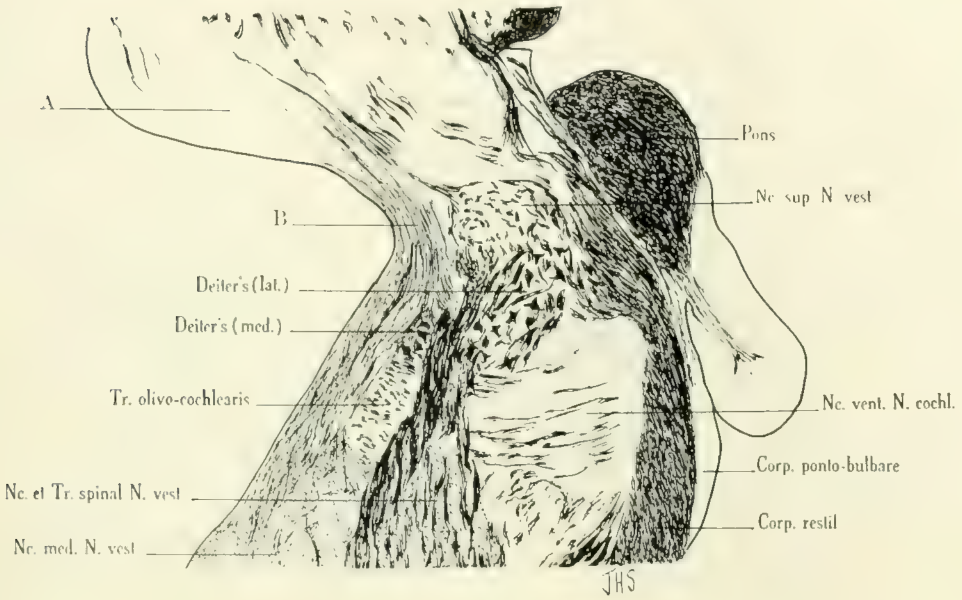


Fig. 10 Horizontal section somewhat above the level of the bifurcation of the fibers of the right radix N. vestibularis into ascending and descending roots. Series B, slide 75, section 1. Enlarged 10 diameters. *A*, ganglion mass connected apparently with the nucleus medialis N. vestibularis (see fig. 11); *B*, marks the fibers connecting the two; *C*, possible cochlear-vestibular connection. For description of section, see page 429.



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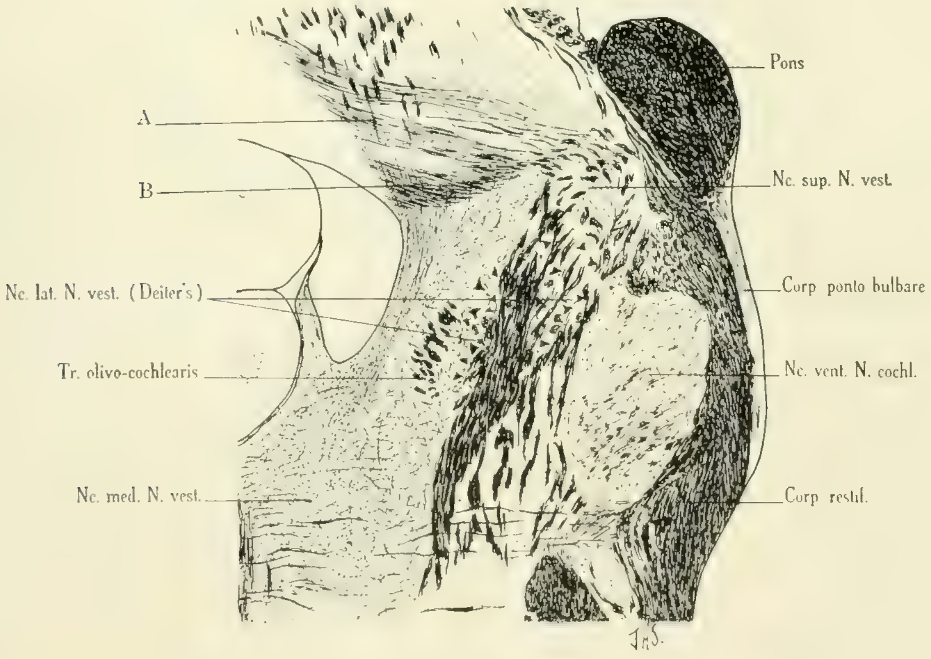

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Fig. 11 Horizontal section taken somewhat ventral to that in fig. 10, showing the relation of Deiter's nucleus to the radix descendens N. vestibularis. Series B, slide 79, section 2. Enlarged 10 diameters. *A* and *B*, as in the preceding figure, represent a possible connection of the medial vestibular nucleus with the central grey substance. For description of section, see page 430.

Fig. 12 Horizontal section at the level of the decussation of fibers from Bechterew's nuclei. Series B, slide 82, section 2. Enlarged 10 diameters. For description of section, see page 431.

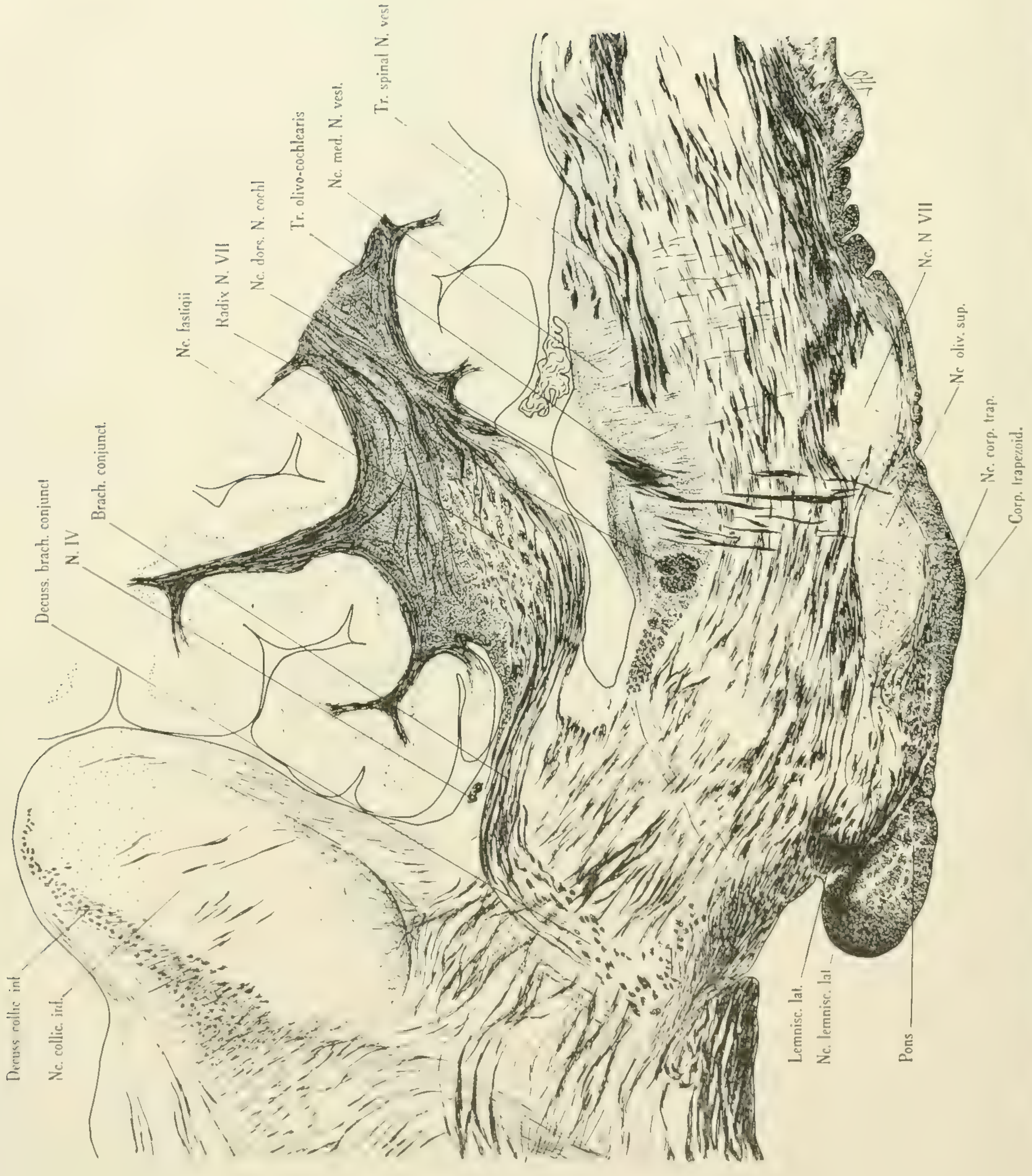

Fig. 13 Sagittal section taken midway between the raphe and the lateral surface of the brain stem. Series C, slide 80, section 2. Enlarged 10 diameters. For description of section, see page 432.

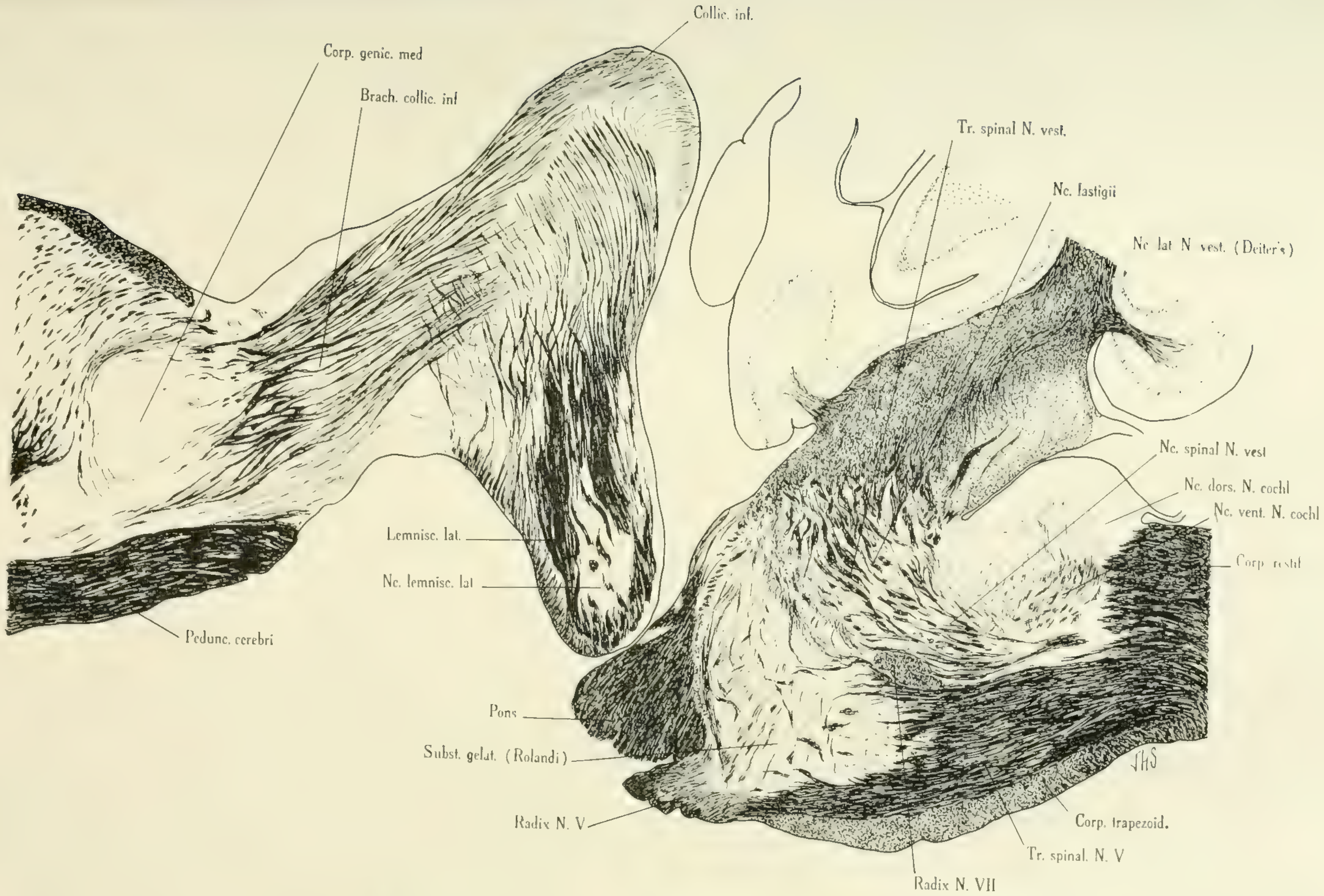

Fig. 14 Sagittal section through the corpus geniculatum mediale, the lemniscus lateralis and the tractus spinalis N. vestibularis. Series C, slide 93, section 1. Enlarged 10 diameters. For description of section, see page 432.

ON THE PRESENCE OF PERMANENT COMMUNICATIONS BETWEEN THE LYMPHATIC AND THE VENOUS SYSTEM AT THE LEVEL OF THE RENAL VEINS IN ADULT SOUTH AMERICAN MONKEYS[1]

CHARLES F. SILVESTER

From the Laboratory of Comparative Anatomy, Princeton University

TWELVE FIGURES

The writer has examined eighty-nine adult monkeys, including both Old World and South American species, which have been added to the Princeton Collections during the past two years. In all of these specimens the lymphatic system was injected with colored gelatin solutions, and, in the majority of cases, the injection was made into the inguinal, mesenteric, axillary, and cephalic lymph nodes.

The following observation was made during the course of this investigation: *Whenever the mesenteric or inguinal lymphatic nodes of a New World species were injected the injection mass never passed from the lumbar or intestinal lymphatic trunks into the thoracic duct or into the anterior regions of the body, but passed directly into the postcava in the region of the renal veins.* A more detailed examination of the vessels in this region of the body revealed the fact *that the lymphatics of the digestive organs and of the posterior extremities invariably enter the venous system at the level of the renal veins.*

Twenty-five individuals, comprising seven of the twelve genera of South American monkeys, form the basis of the present investigation. These posterior communications between the lymphatic and the venous system were found to vary from two to nine in number and were found to open at almost any point on the renal segment of the postcava and its immediate tributaries.

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[1] Read before the twenty-fifth Session of the Association of American Anatomists; Boston, Mass., December 28 to 30, 1909, and the II International Anatomical Congress; Brussels, August 7 to 11, 1910.

Sixteen different species of Old World monkeys have been examined and no indication of these reno-caval communications have been found. Their uniform presence in the New World forms is a fact which further serves to establish the wide separation supposed to exist between these two groups of Primates.

The species which form the material for the present paper belong to the families Cebidae and Callitrichidae, and are distributed among seven of the twelve genera of New-World monkeys.

The following table[2] shows the genera and species examined. Genera indicated in italics have not been examined.

Family Cebidae	NUMBER OF INDIVIDUALS EXAMINED
Alouata seniculus	1
Ateles variegatus	1
Ateles ater	2
Ateles hybridus	2
Ateles vellerosus	1
Brachyteles	0
Lagothrix	0
Cebus hypoleucus	10
Cebus capucinus	3
Pithecia	0
Cothurus	0
Callicebus	0
Saimiris sciurea	1
Nyctipithecus trivirgatus	1
Family Callitrichidea	
Callithrix jacchus	1
Midas oedipus	2

THE VEINS

The postcava and its tributaries present the normal Primate arrangement. The left renal vein, as a rule, enters the postcava somewhat cranial to the right, although the left kidney is usually situated slightly caudad of the right kidney. This, as well as the fact that the postcava is developed on the right side of the aorta, necessitates an increase in the length of the left renal vein. The left suprarenal and sex veins open into the corresponding renal; while those on the right side of the body enter the postcava direct, the suprarenal above, and the sex vein below the renal vein. Two small phrenic veins from the pillars of the diaphragm usually enter the postcava directly caudad of the hepatic tributaries. These veins may at times, however, enter the renal or suprarenal veins.

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[2] Nomenclature taken from E. L. Trouessart, Catalogus Mammalium, 1904.

In one specimen of Cebus hypoleucus (plate 5) the normal position of the veins was reversed, the postcava being situated on the *left* side of the aorta. This was the only radical variation from the normal position of the postcava which was met with in an examination of the venous system of a very large number of monkeys. It is interesting to note that in this individual the morphological position of the veno-lymphatic communications was not changed, though these communications were of course shifted to the opposite side of the postcava. This variation in the position of the postcava and the resultant shifting of the lumbar lymphatic trunks, illustrates very beautifully the reciprocal relationship between the veins and the lymphatics. Huntington and McClure[3] have pointed out the reason for this relationship between the veins and the lymphatics. This is very clearly expressed by Dr. Huntington[4] in a review of the above work. "The developmental shifting of the primitive redundant embryonal venous system from the original bilateral symmetrical type to the dextro-venous condition of the adult favors the sinistral development of the main lymphatic channels, which replaces the atrophied left segment of the primitive bilateral symmetrical venous system of earlier embryonal stages." Naturally in this variation in the position of the postcava one would expect to meet with a corresponding variation in the position of the lymphatics.

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[3] George S. Huntington and C. F. W. McClure, The Development of the Main Lymph Channels of the Cat in their Relations to the Venous System, Am. Jour. of Anat., vol. 6, 1907.

[4] George S. Huntington, The Genetic Interpretation of the Development of the Mammalian Lymphatic System, Anat. Record, vol. 2, 1908.

THE LYMPHATICS

The reno-caval lymphatico-venous communications naturally fall under two heads: (1) those between the intestinal lymphatic trunks and the veins and; (2) those between the lymphatics from the posterior extremities and lumbar regions and the veins. They will be considered in this order.

Text-fig. 1 indicates the position and number of all the reno-postcaval communications found in the twenty-five individuals examined. No attempt has been made in this figure to show a separation between the area occupied by the mesenteric and that occupied by the lumbar communications, for the reason that in many instances the two areas overlap. In general, however, it may be stated, that the mesenteric area of communication is represented by the circles situated on the cranial border of the left renal vein and on the left side of the postcava, and that the remainder of the circles represent the area covered by the lumbar communications.

1. *Communications between the intestinal lymphatic trunks and the veins.* The communications between the intestinal lymphatic trunks and the veins were found to vary in number in different individuals, from one to four. In the majority of individuals two main trunks are present, one situated on either side of the mesenteric artery. The trunk on the left side of the mesenteric artery usually opens into the left renal vein, while the one on the right side, as a rule, opens into the left side of the postcava, cranial to the renal vein. The above might be designated as the typical arrangement, if a typical arrangement can be said to exist, and is shown in fig. 4 (Cebus hypoleucus), and fig. 8 (Saimiris sciurea). In some cases the two mesenteric trunks both open into the left renal vein (No. 2465, Cebus capucinus), or both open into the postcava cranial to the renal vein (fig. 1, Alouata seniculus and fig. 9, Callithrix jacchus).

Many variations from this double-trunk arrangement were met with. The intestinal trunk may be single, with a single opening into the postcava, as in Cebus capucinus (2481),[5] and Nycti-

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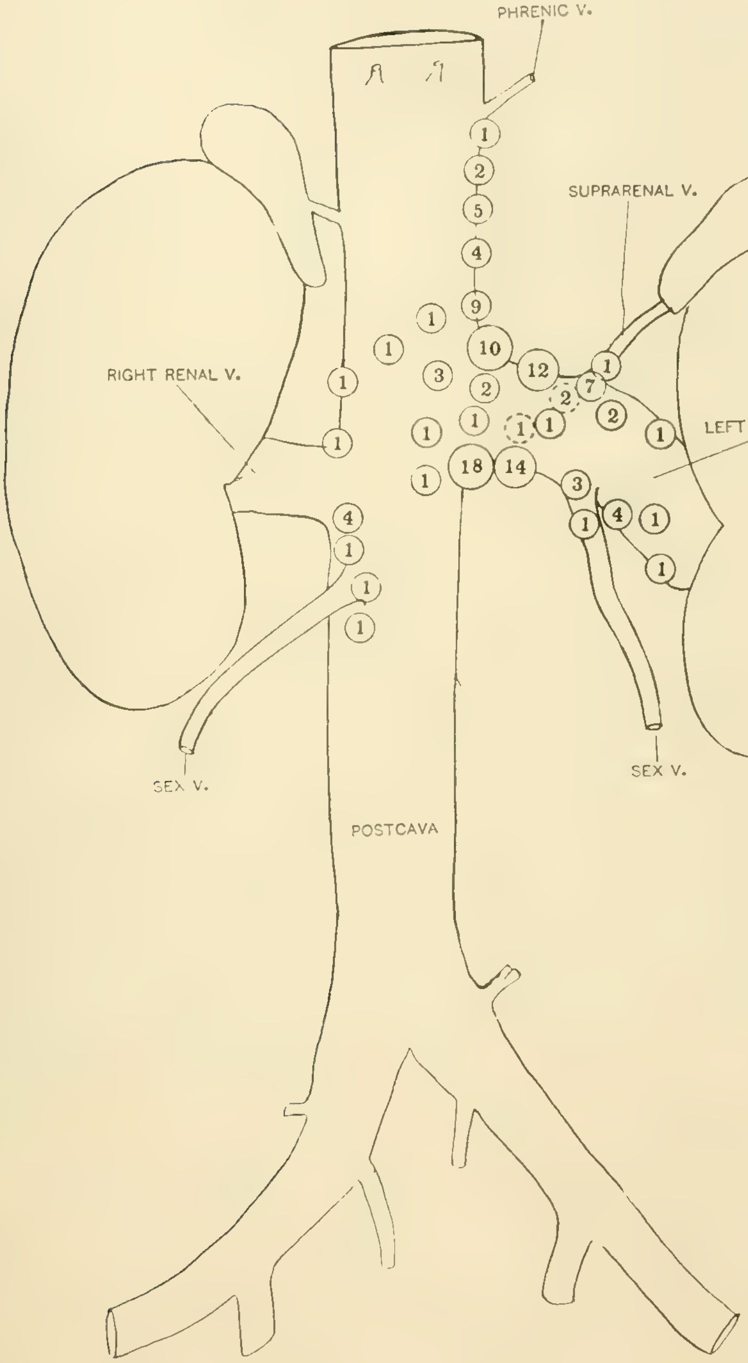
[5] The number after the name of the species refers to the catalogue number of the individual in the Princeton Collection.



Text-fig. 1 Showing the position and number of the Reno-caval communications found in the twenty-five individuals examined. The circles indicate the positions of the communications and the figures in the circles indicate the number of communications found in each position.

pithecus trivirgatus, or with a single opening into the left renal vein, as was the case in one specimen of Ateles atar. The single intestinal trunk may open in common with the right lumbar trunk into the cranial surface of the left renal vein (fig. 6, Cebus hypoleucus), or with the left lumbar trunk into the caudal surface of the left renal vein (Cebus hypoleucus, 2474). In one specimen of Midas oedipus (fig. 10) the single truncus intestinalis split into three branches and opened, one branch into the ventral surface of the postcava, the other two into the left renal vein. The intestinal lymphatics may form a network with as many as four openings into the veins (Ateles vellerosus, fig. 3 and Ateles variegatus, fig. 2) There may be three trunks present, two opening into the postcava and one into the renal vein (No. 2493, Cebus hypoleucus), or two into the left renal vein and one into the postcava (Cebus hypoleucus, 2471).

In many instances anastomoses occur between the mesenteric and the lumbar lymphatic trunks. These are shown in their more complicated form in fig. 2 (Ateles variegatus). A simple joining of the two trunks, forming what may be termed a mesenterico-lumbar communication, occurred in one specimen of Cebus hypoleucus (2474). In this individual the mesenteric trunk divides on each side of the left sex-vein and opens with the left lumbar trunk into the caudal surface of the left renal vein.

2. *Communications between the lumbar lymphatic trunks and the veins.* The lymphatico-venous communications of the vessels from the posterior extremities and lumbar regions present quite as many, or even more variations in regard to position and number than do those of the mesenterics. They vary in number from two to as many as five openings and may be situated at almost any point or combination of points on the veins of the renal region. The variations in the position and number of these communications were found to be so great that the writer has been unable to find any two individuals possessing the same arrangement. In general it might be stated that the lumbar trunks of the right side open either into the postcava, or at the juncture of the postcava and the left renal vein, and that those of the left side open into the left renal vein.

There are three general points of communication which were found to be more or less constant in position, and although these three points were found to be present, to the exclusion of all others, in only one individual (fig. 4, Cebus hypoleucus), this might be designated as the typical arrangement for the reason that at least one of these three openings was present in every individual examined. These three points of communication are connected with three lumbar trunks as follows:

(1) A lumbar trunk from the left side and opening into the left renal vein near the sex-vein; (2) a lumbar trunk coming from the right side, crossing the ventral surface of the postcava, and opening at the juncture of the left renal vein and the postcava; and (3) a trunk from the right side opening into the postcava near the right renal or right sex-vein. The first two communications were present in more than 75 per cent of the individuals examined. The third was not nearly so constant; it represents, however, the only trunk opening on the right side.

The lumbar trunks of the two sides may join and have a common opening, either into the left renal vein (Cebus capucinus, No. 2559), or at the juncture of the left renal vein and the postcava (Midas oedipus, fig. 10). As a rule, however, the trunks of the two sides have separate openings into the veins. They remain distinct except for numerous cross anastomoses which occur between the two sides (figs. 1 and 7). Anastomoses between the lumbar and mesenteric trunks, as mentioned above, are also of common occurrence (figs. 2 and 7, Ateles variegatus, 2563, Cebus hypoleucus, 2574).

As mentioned above, the lumbar trunks of the two sides, as a rule, have separate openings into the veins. For this reason the right and left lumbar trunks are considered separately.

a. The right lumbar lymphatic trunks. The lumbar lymphatic vessels of the right side may present a single communication on the right side of the postcava, either cranial to the renal vein (Cebus hypoleucus, No. 2480), or caudal to or at the level of the renal vein (figs. 4 and 7, Cebus hypoleucus). The right trunks may also open on the left side of the postcava in similar relations to the left renal veins (figs. 3 and 9, Ateles vellerosus and Callithrix jacchus). The single opening most frequently met with is at the juncture of the left renal vein and the postcava (fig. 2, Ateles variegatus, 2563). In one individual of Cebus hypoleucus (fig. 6) the right lumbar trunk joined with the intestinal lymphatic trunk to open into the cranial surface of the left renal vein. In about 50 per cent of the individuals the right lumbar lymphatic trunks possess multiple communications; one may be situated at the juncture of the left renal vein and the postcava, and the other on the ventral surface of the postcava near the right renal vein (fig. 4, Cebus hypoleucus, 2472), or one of these openings may be situated on the dorsal surface of the left renal vein (fig. 8, Saimiris sciurea). In one individual of Ateles vellerosus (fig. 3) the trunks from the right side communicated with the veins at three points, two on the left renal vein and one on the ventral surface of the postcava.

b. The left lumbar lymphatic trunks. The communications of the left lumbar trunks do not possess so wide a variation in position. They have, however, a greater variation in number than do those of the right side. They may be single (figs. 4 and 6, Cebus hypoleucus), or as many as four in number No. 2465 Cebus capucinus). In the majority of individuals the lumbar lymphatic trunks from the left side of the body communicate with the left renal vein. In certain cases, however, these trunks all open into the postcava near the renal vein (fig. 8, Saimiris sciurea); or by a single aperture cranial to the renal vein (fig. 9, Callithrix jacchus); or they may terminate by two openings on the left renal vein and two on the postcava (Ateles hybridus, 2433).

An interesting variation is met with in Nyctipithecus trivirgatus. In this individual the right and left lumbar lymphatic trunks join together and open by three communications into the postcava cranial to the left renal vein.

TABLE 1

Table indicating the species and individuals examined and the position and number of all Reno-Caval communications

INDIVIDUALS EXAMINED	MESENTERIC COMMUNCIATIONS		LUMBAR COMMUNICATIONS		TOTAL NUMBER OF RENO-POSTCAVAL COMMUNICATIONS
	No.	Position	No.	Position	
Alouata seniculus, Linn. ♂ No. 2463 (Pl. 1)	2	Postcava cranial to left renal V. and at juncture of left renal V. and postcava	3	Left renal V., at juncture of left renal and postcava. and anastomoses with mesenteric trunk	4
Ateles variegatus, Wag. No. 2563 (Pl. 2)	4	Left renal V. and postcava at juncture of renal V. together with right lumbar trunk	6	Left renal V. and postcava at juncture of left renal V.	7
Ateles vellerosus, Gray. ♀ No 2476 (Pl. 3)	4	Left renal V. and postcava cranial to renal V.	5	Left renal V., ventral surface of postcava, and at juncture of postcava and left renal V.	9
Ateles ater, Cuvier. Columbia Univ. No. 696	1	Cranial surface of left renal V.	4	Left renal V. and postcava at juncture of left renal V.	5
Ateles ater, Cuvier. Columbia Univ. No. 842	1	At juncture of left renal V. and postcava with right lumbar trunk	2	Left renal V. and at juncture of left renal V. and postcava	3
Ateles hybridus, Geoff. ♂ No. 2433	1	Ventral surface of postcava near left renal V.	5	Left renal V., ventral surface of postcava, and postcava near right renal V.	5
Ateles hybridus, Geoff. ♂ No. 2435	2	Left renal vein and postcava cranial to left renal V.	4	Left renal V. at juncture of postcava and left renal and postcava near right renal V.	6
Cebus hypoleucus, Humb. ♀ No. 2471	3	Left renal V. and postcava cranial to left renal V.	2	Left renal V. and at juncture of left renal V. and postcava	5

TABLE 1—Continued

INDIVIDUALS EXAMINED		MESENTERIC COMMUNICATIONS		LUMBAR COMMUNICATIONS		TOTAL NUMBER OF RENO-POSTCAVAL COMMUNICATIONS
		No.	Position	No.	Position	
Cebus hypoleucus, Humb. No. 2472 (Pl. 4)	♀	2	Left renal V. and postcava cranial to left renal V.	3	Left renal V., right side of postcava, and postcava at juncture of left renal V.	5
Cebus hypoleucus, Humb. No. 2473	♀	2	Left renal V. and postcava cranial to left renal V.	2	Left renal V. and right side of postcava caudal to renal V.	4
Cebus hypoleucus, Humb. No. 2474	♀	1	Left renal V. with left lumbar trunk	2	Left renal V. and postcava cranial to right renal V.	2
Cebus hypoleucus, Humb. No. 2475 (Pl. 5)	♂	2	Postcava cranial to left renal V. (Postcava on left of aorta)	2	Postcava near right spermatic and renal V.	4
Cebus hypoleucus, Humb. No. 2480	♂	1	Postcava cranial to left renal V.	2	Left renal V. and ventral surface of postcava opposite left renal V.	3
Cebus hypoleucus, Humb. 2486 (Pl. 6)	♀	1	Left renal V.	2	Left renal V.	2
Cebus hypoleucus, Humb. No. 2493	♂	3	Left renal V. and postcava cranial to left renal V.	4	Left renal V. and at juncture of left renal vein and postcava.	7
Cebus hypoleucus, Humb. No. 2540	♀	2	Left renal V. and postcava cranial to left renal V.	3	Left renal V., left spermatic V. and at juncture of left renal and postcava	4
Cebus hypoleucus, Humb No. 2574 (Pl. 7)	♀	2	Left renal V. and at cranial juncture of left renal and postcava	4	Left renal V., at juncture of left renal and postcava, ventral surface of postcava, and postcava cranial to right renal V.	6

TABLE 1—Continued

Cebus capucinus, Linn. ♀ No. 2465	2	Left renal V.	5	Left renal V. and postcava near left renal V.	7
Cebus capucinus, Linn. ♀ No. 2481	1	Postcava cranial to left renal V.	3	Left renal V. and postcava near left renal V.	4
Cebus capucinus, Linn. No. 2559	3	Left renal V., postcava cranial to left renal V., and joins lumbar trunk	2	Left renal V.	4
Saimiris sciurea, Linn. ♀ No. 2554 (Pl. 8)	2	Dorsal surface of left renal V. and ventral surface of postcava near renal V.	3	Dorsal surface of left renal V., postcava caudal to left renal and postcava opposite left renal V.	5
Nyctipithecus trivirgatus. Columbia Univ. No. 805	2	Postcava cranial to left renal V.	3	Postcava cranial to left renal V.	4
Callithrix jacchus, Linn. No. 2537 (Pl. 9)	2	Postcava cranial to left renal V.	2	Postcava cranial to left renal V.	4
Midas oedipus, Linn. ♀ No. 2249	4?	Postcava adjacent to left renal V. (Not injected)	2?	Postcava cranial to renal V. (Not injected).	6?
Midas oedipus, Linn. ♂ No. 2773 (Pl. 10)	3	Left renal V. and postcava near left renal V.	1	Postcava directly caudal to left renal V.	4

In speaking of lymph-heart formations, Dr. Huntington, in his most interesting paper "The Genetic Interpretation of the Development of the Mammalian Lymphatic System,"[4] makes the statement that the phylogenetic history of these structures permits of their presence at the posterior as well as at the anterior region of the body. However, except for their presence in the cervical region, as is usually the case in mammals, similar structures have not been recognized up to the present time in other parts of the body as being carried into the adult organization as portals of lymphatico-venous entry.

The constant occurrence of communications at the posterior region of the body between the lymphatic and the venous system has not hitherto been shown to exist in an adult mammal.

Huntington and McClure[6] and Lewis[7] have shown that the permanent communications in the jugular region between the jugular lymph-sac and the systemic veins appear to be formed secondarily. Although a proper interpretation of the reno-caval communications in South American monkeys is one which can only be determined on embryological evidence, it is natural to suppose that their number and position in the adult should more or less conform to primary connections present in the embryo. This has been shown to be the case in the anterior region of the body for mammals in general in a joint paper by Professor McClure and the writer.[8]

The mammalian lymphatic system as generally understood is represented in *B*, text-fig. 2, in which only one drainage center is present. The thoracic duct is situated on the right side of the aorta and opens on the left side of the body at the jugular or subclavian angle.

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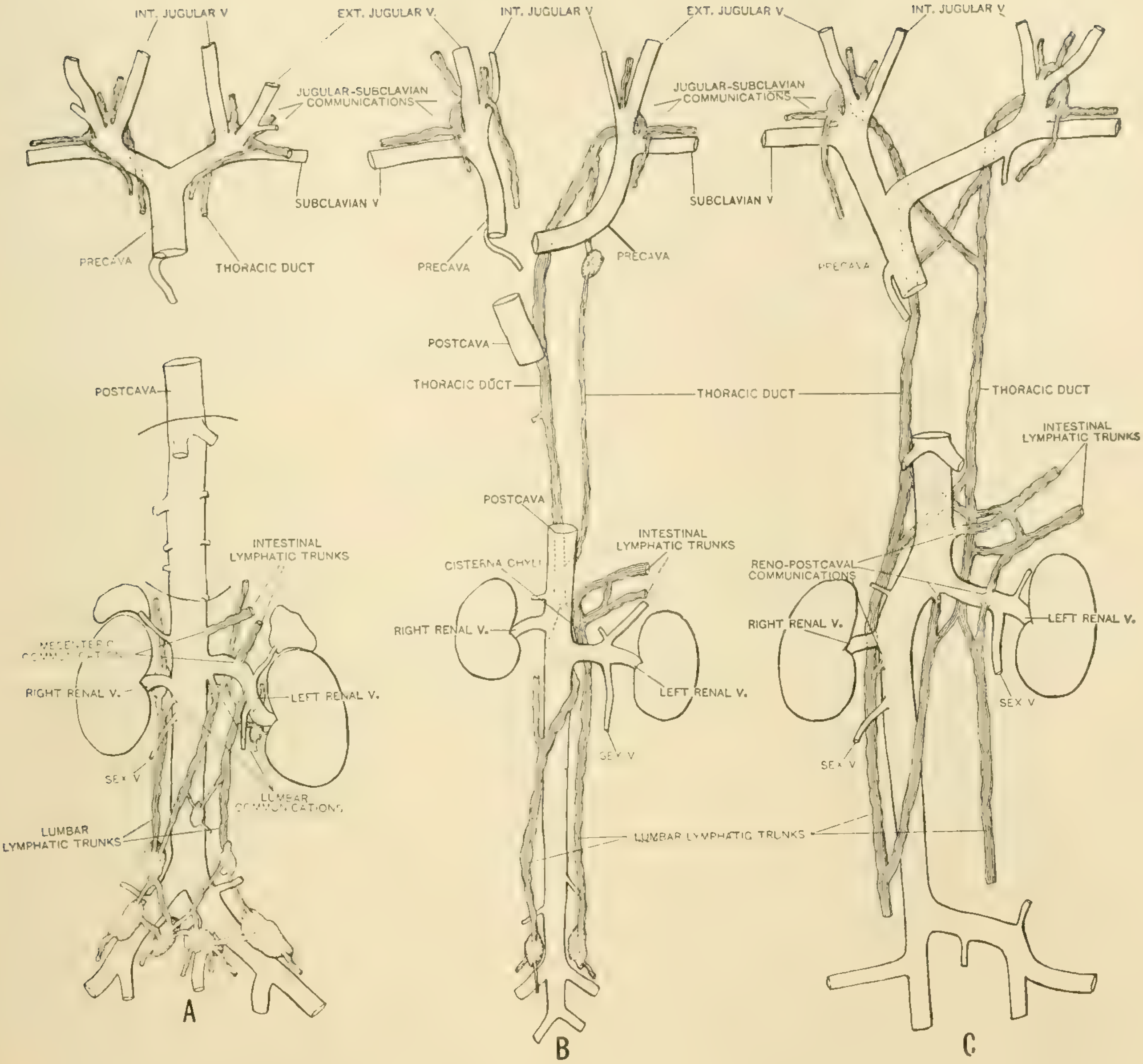
[6] George S. Huntington and Charles F. W. McClure, The Anatomy and Development of the Jugular Lymph Sacs in the Domestic Cat (Felis Domestica), Anat. Record, vol. 2, 1908.

[7] Frederick T. Lewis, The Development of the Lymphatic System in Rabbits, Am. Jour. of Anat., vol. 5, 1905.

[8] Charles F. W. McClure and Charles F. Silvester, A Comparative Study of the Lymphatico-Venous Communications in Adult Mammals, Anat. Record, vol. 3, 1909.



Text-fig. 2 Diagrams illustrating the main lymphatic trunks and their relations to the veins: *A*, South American monkeys (drawing modified from *Cebus capucinus*, 2481): *B*, Mammals where Reno-caval communications are not present (drawing modified from *Lepus cuniculus*, 2367): and *C*, Mammals in general (compiled from the dissections of the lymphatic system of about eighty-five adult mammals).

Text-fig. 2, *A*, shows the conditions met with in South American monkeys. Here there are two distinct drainage centers, an anterior and a posterior. The anterior, or jugular-subclavian district of communication, drains the thoracic and anterior regions of the body; while the posterior, or reno-postcaval district of communication, drains in general all of that region situated caudal to the thorax. The thoracic and right lymphatic ducts are much reduced in South American monkeys. They drain the heart and lungs and anterior portion of the thorax. The diaphragm and posterior portion of the thorax are drained back into the postcava through recurrent branches of the lumbar trunks.

Text-fig. 2, *C*, is a diagrammatic representation of the lymphatic system of mammals in general; showing the modifications made necessary by the conditions met with in South American Primates. The intestinal and lumbar lymphatic trunks may open by reno-postcaval communications or they may open at the jugular-subclavian district through the thoracic duct. The thoracic duct may be situated on either the right or the left side of the aorta, or it may be double, one situated on each side of the aorta, and with numerous cross connecting branches between the two. As a rule, the thoracic duct opens on the left side of the body; it may, however, communicate with the right side, or with both sides as shown in the diagram.

EXPLANATION OF PLATES 1 TO 10

Figs. 1 to 10, inclusive, were drawn to scale from dissections of adult mammals and represent ventral views of the veins and lymphatic vessels in the renal and lumbar regions.

The veins are drawn in outline, the lymphatics are shaded, and the arteries, when indicated, are cross-lined.

The name of the species represented is given under each figure, while a complete list of the mammals studied in connection with this paper may be found in the table on pages 455-457.

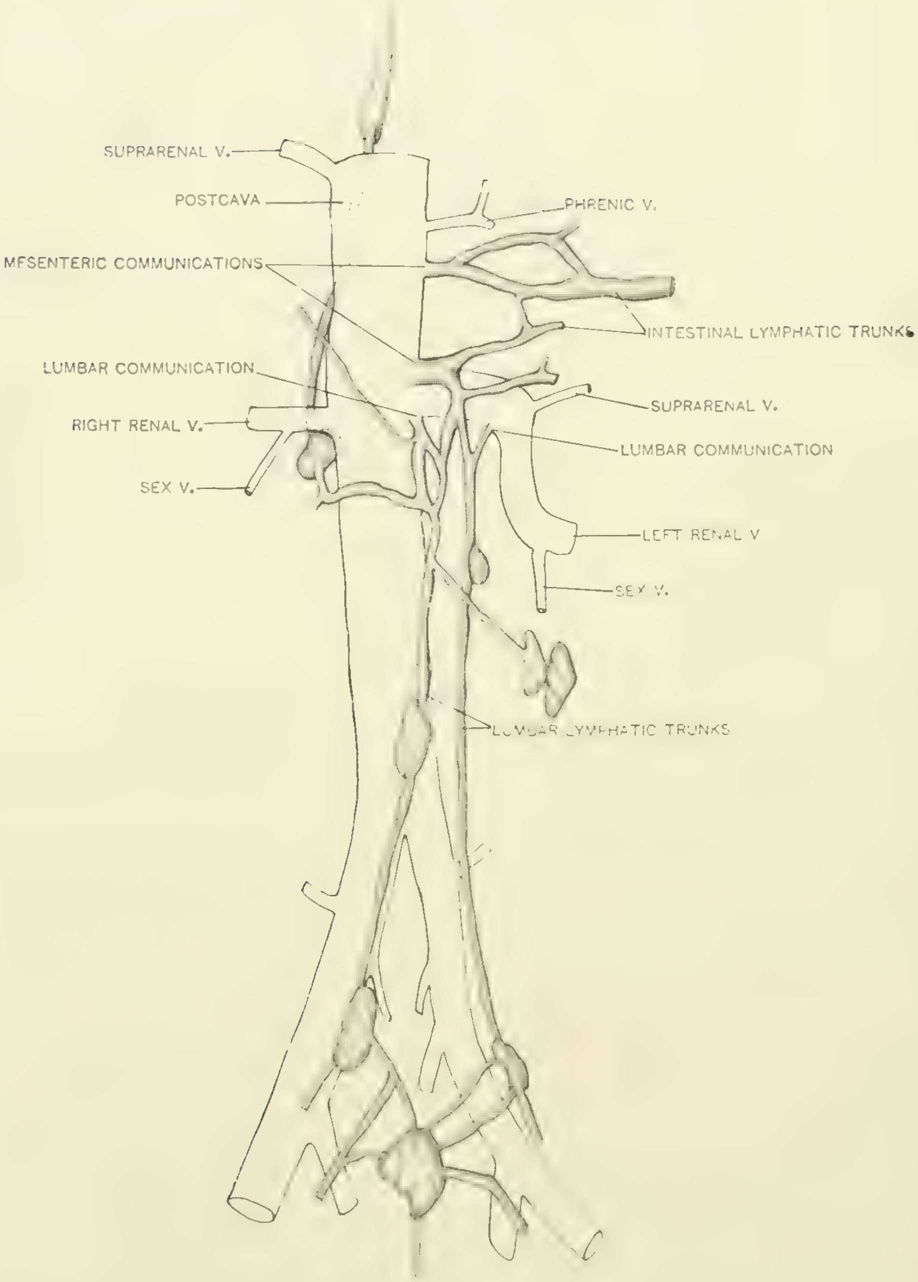


Fig. 1 Alouta seniculas, Linn. Red Howler ♂

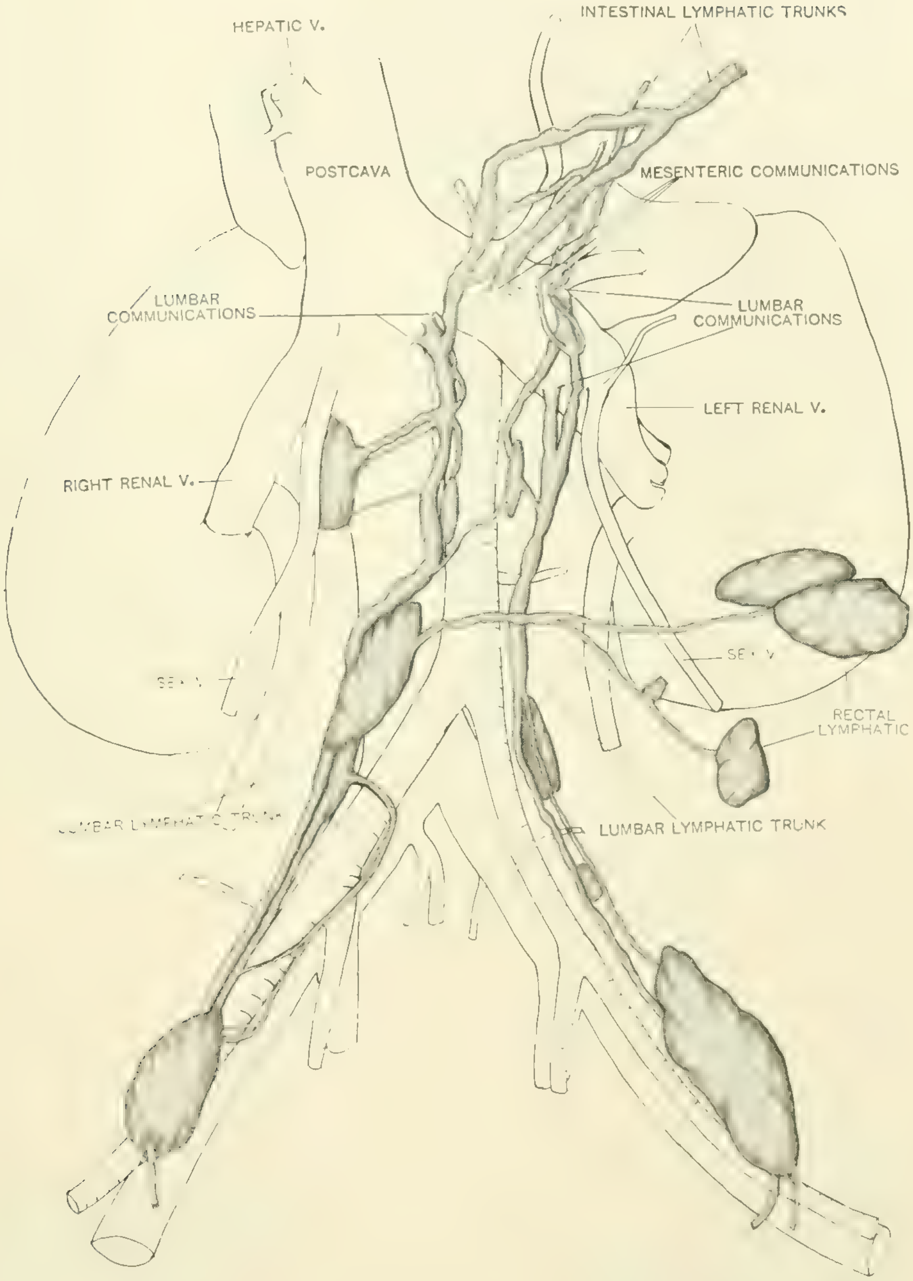


Fig. 2 Ateles variegatus Wagner. Variegated Spider-Monkey

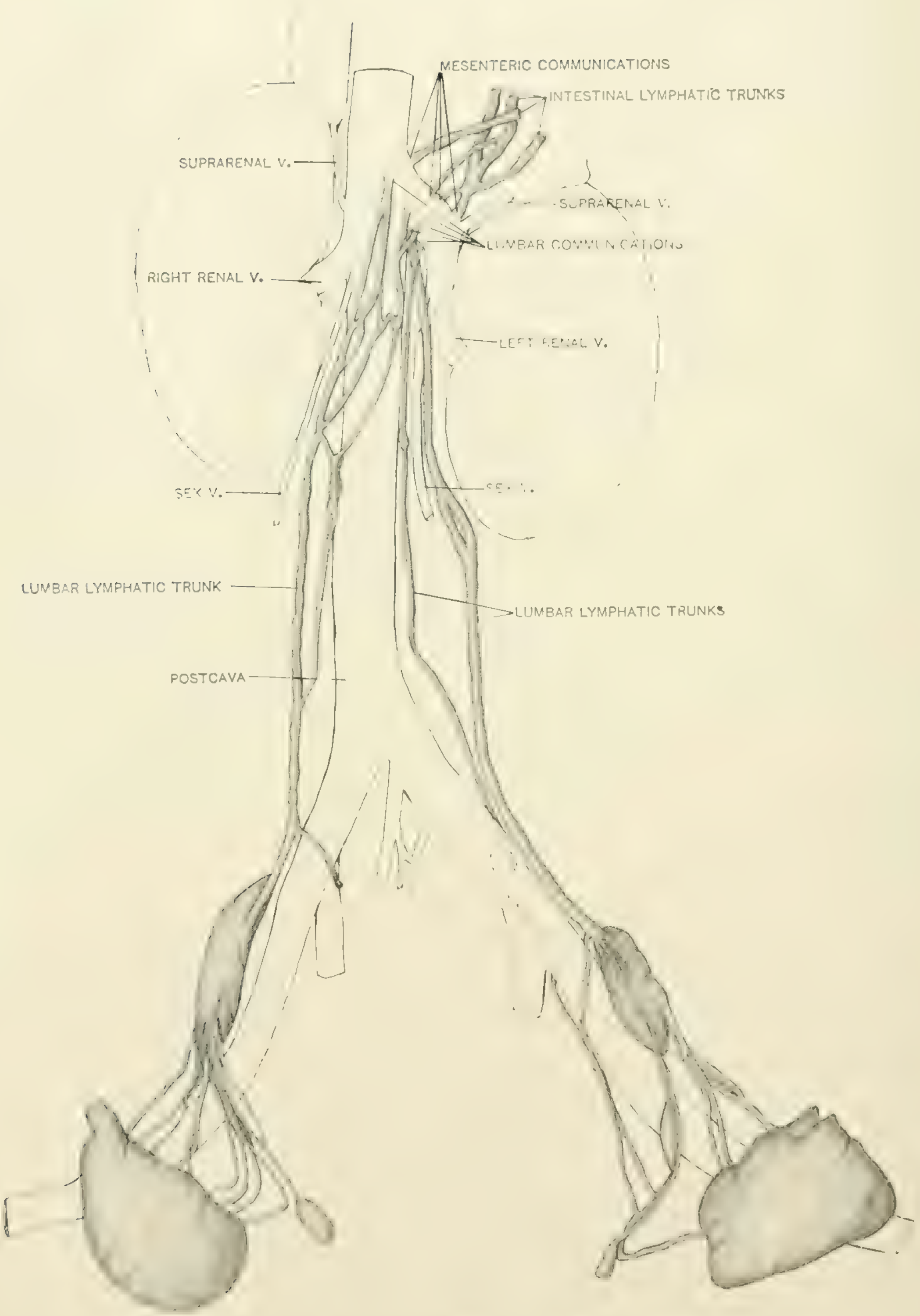


Fig. 3 Ateles vellerosus, Gray. Spider-Monkey ♀

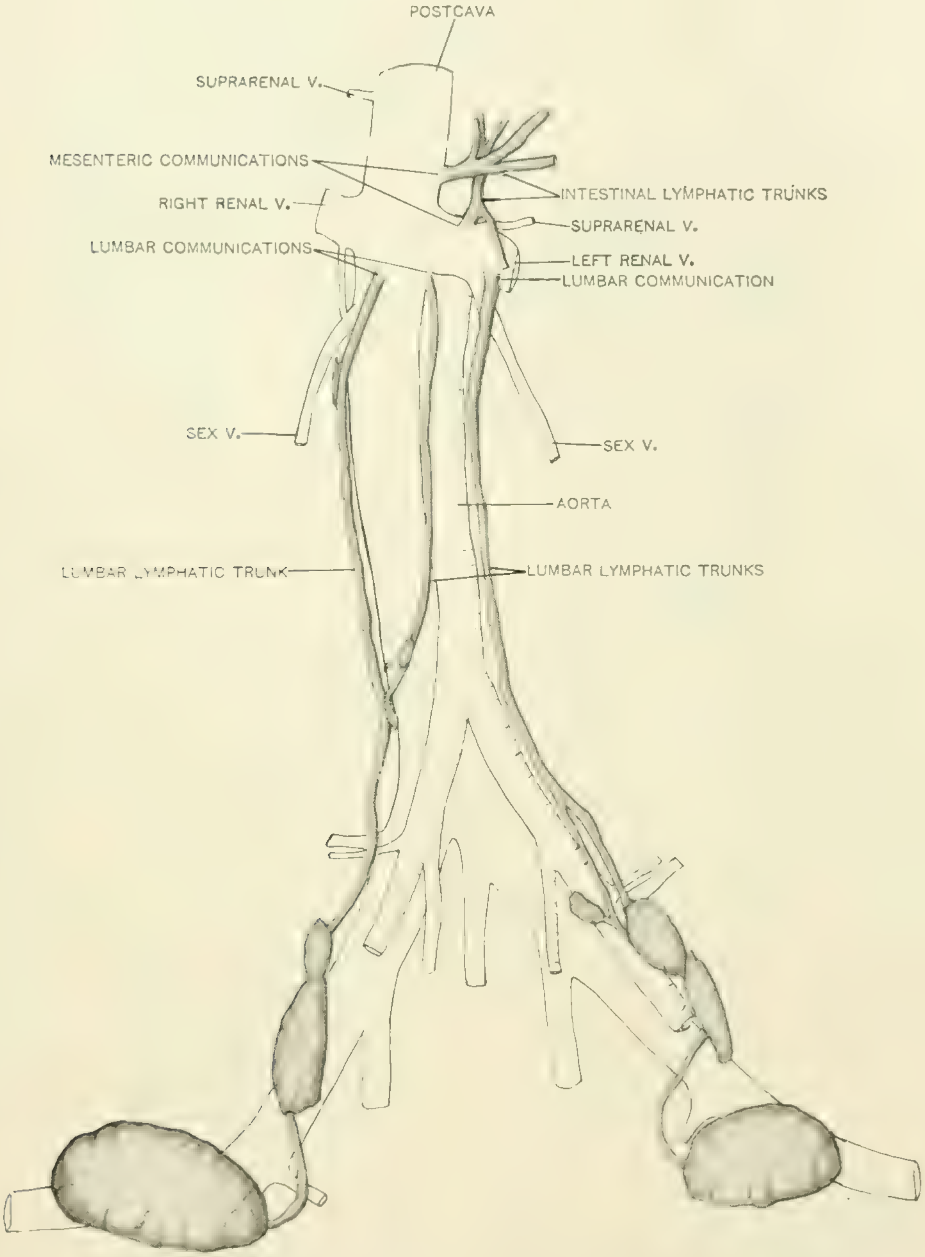


Fig. 4 Cebus hypoleucus, Humb. White-Throated Capuchin ♀

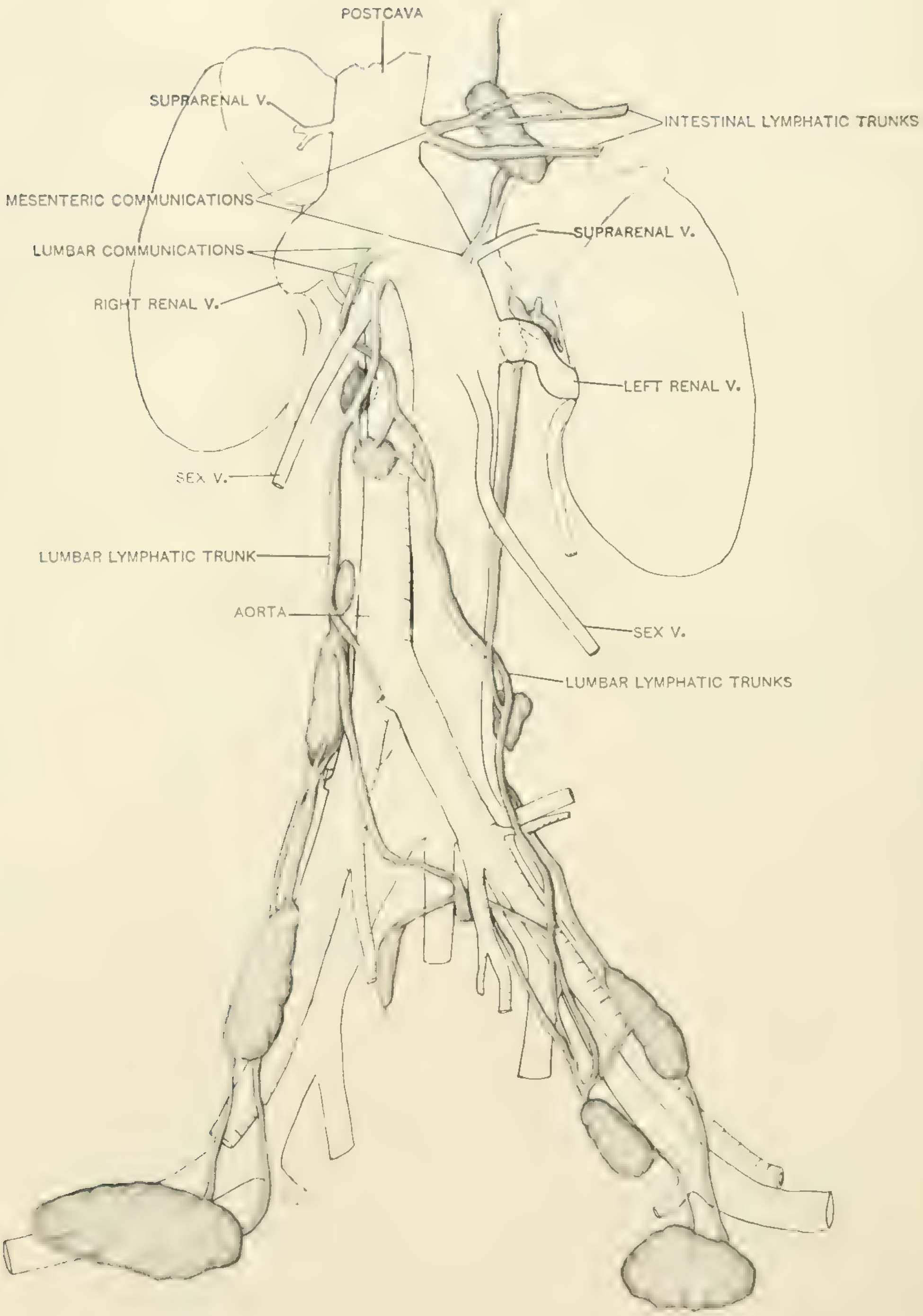


Fig. 5 Cebus hypoleucus, Humb. White-Throated Capuchin ♂

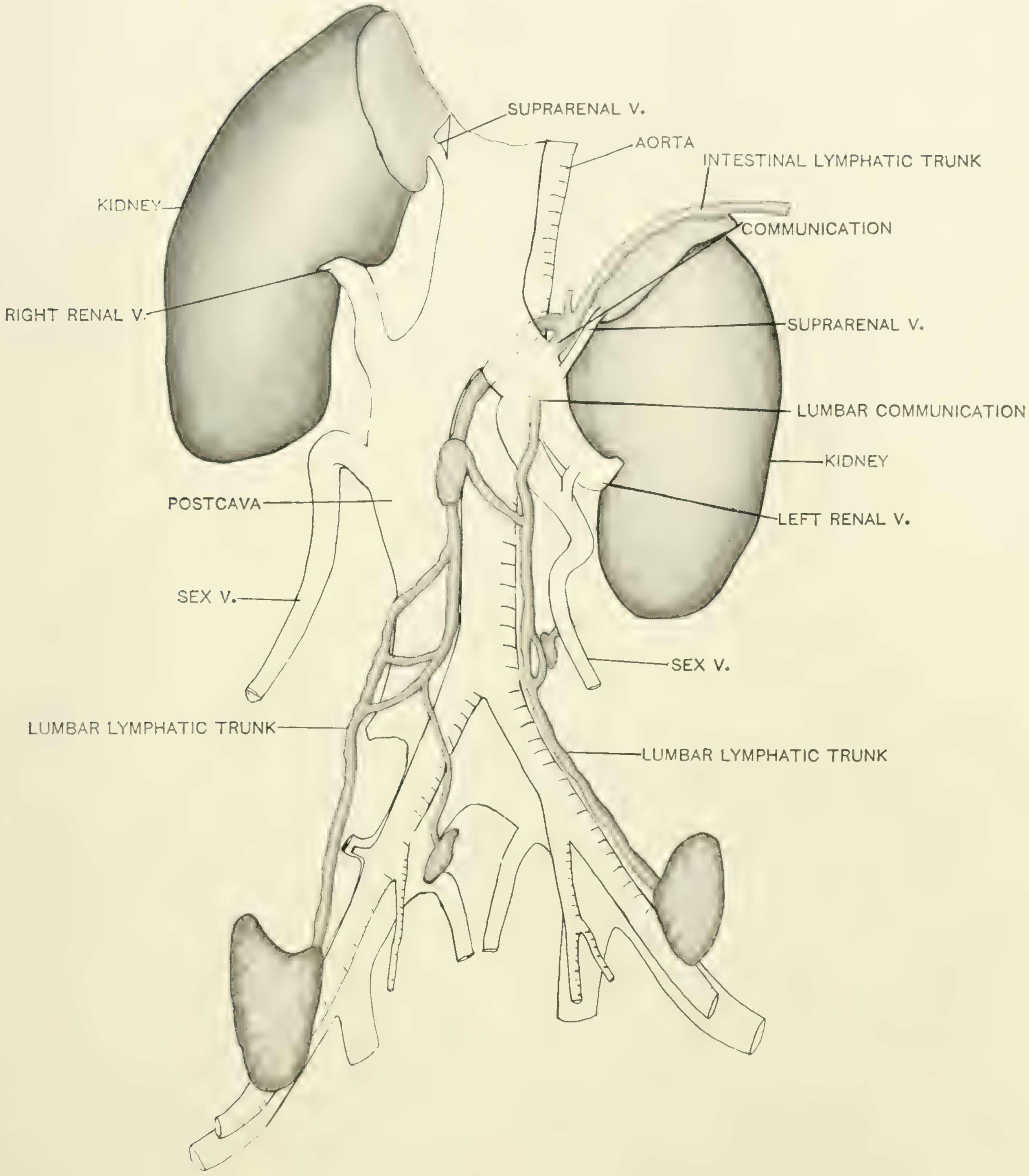


Fig. 6 Cebus hypoleucus, Humb. White-Throated Capuchin ♀

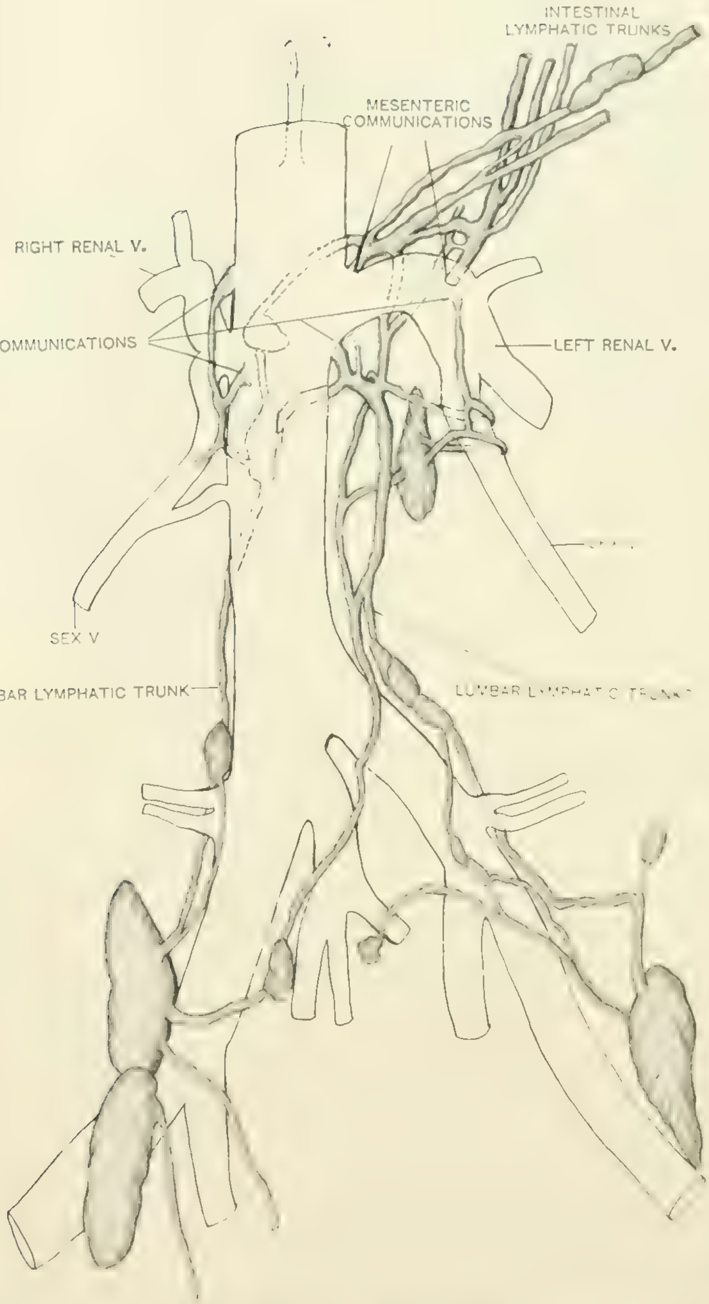


Fig. 7 Cebus hypoleucus, Humb. White-Throated Capuchin ♂

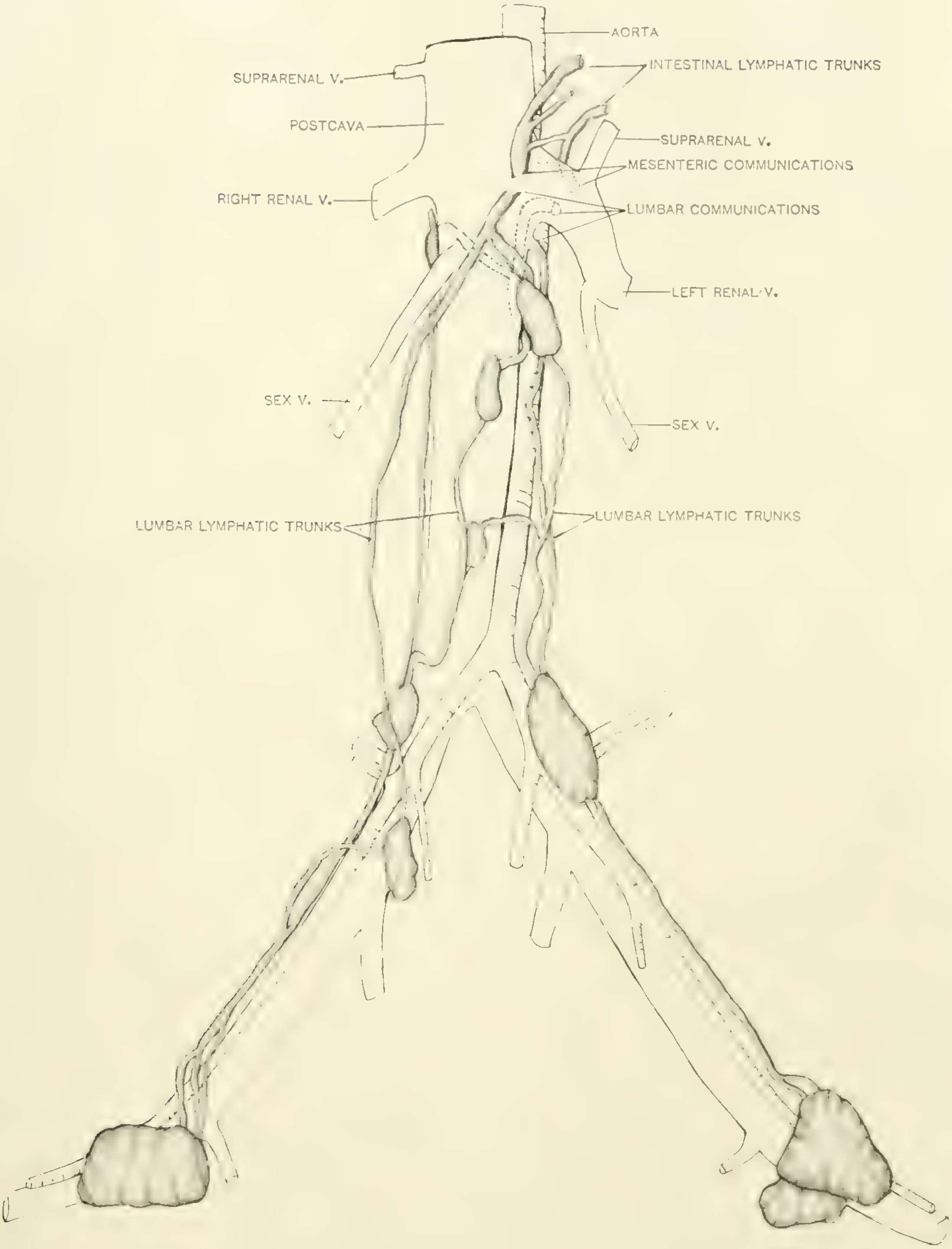


Fig. 8 Saimiris scivrea, Linn. Squirrel-Monkey ♂

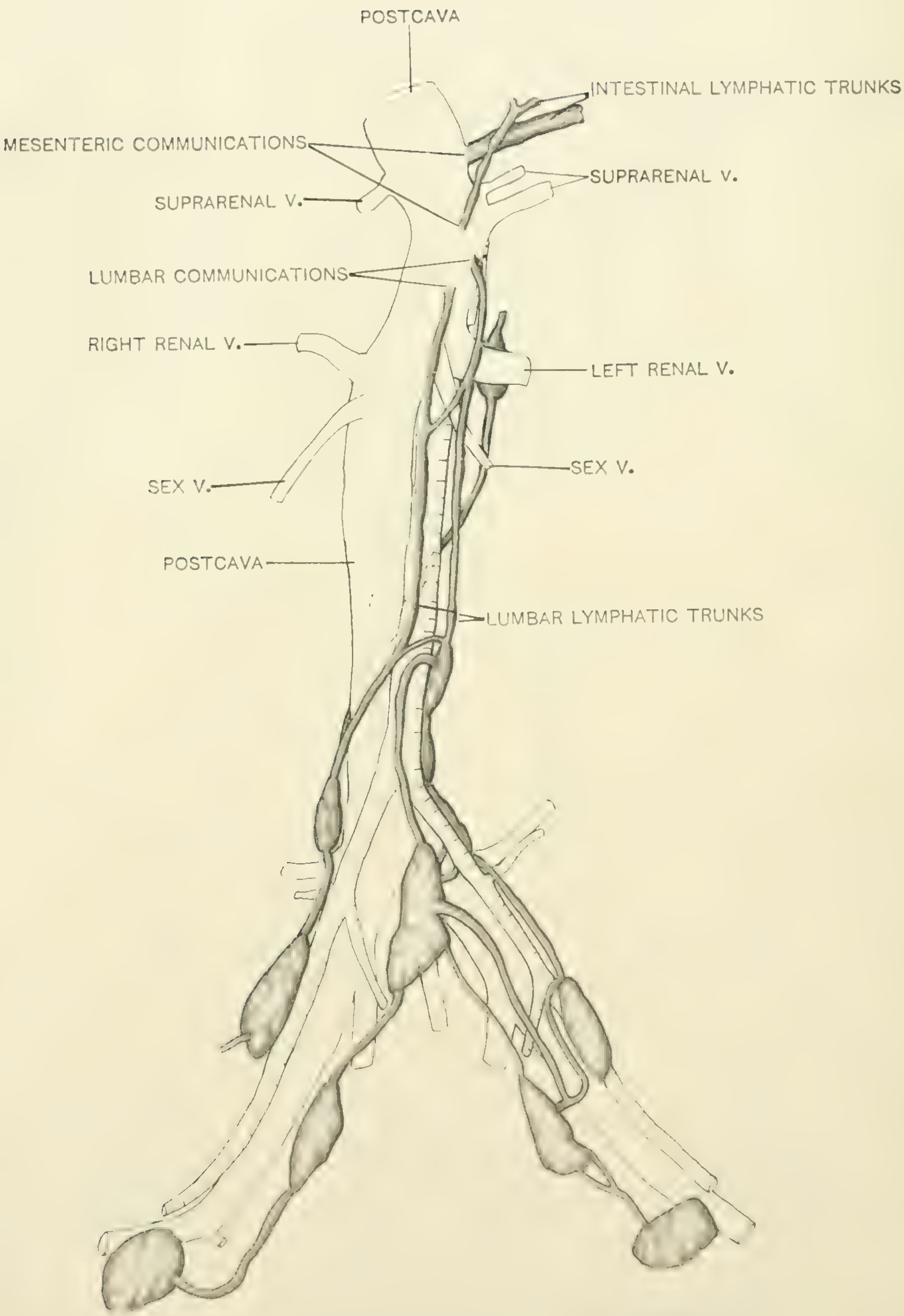


Fig. 9 Callithrix jacchus, Linn. Common Marmoset ♀

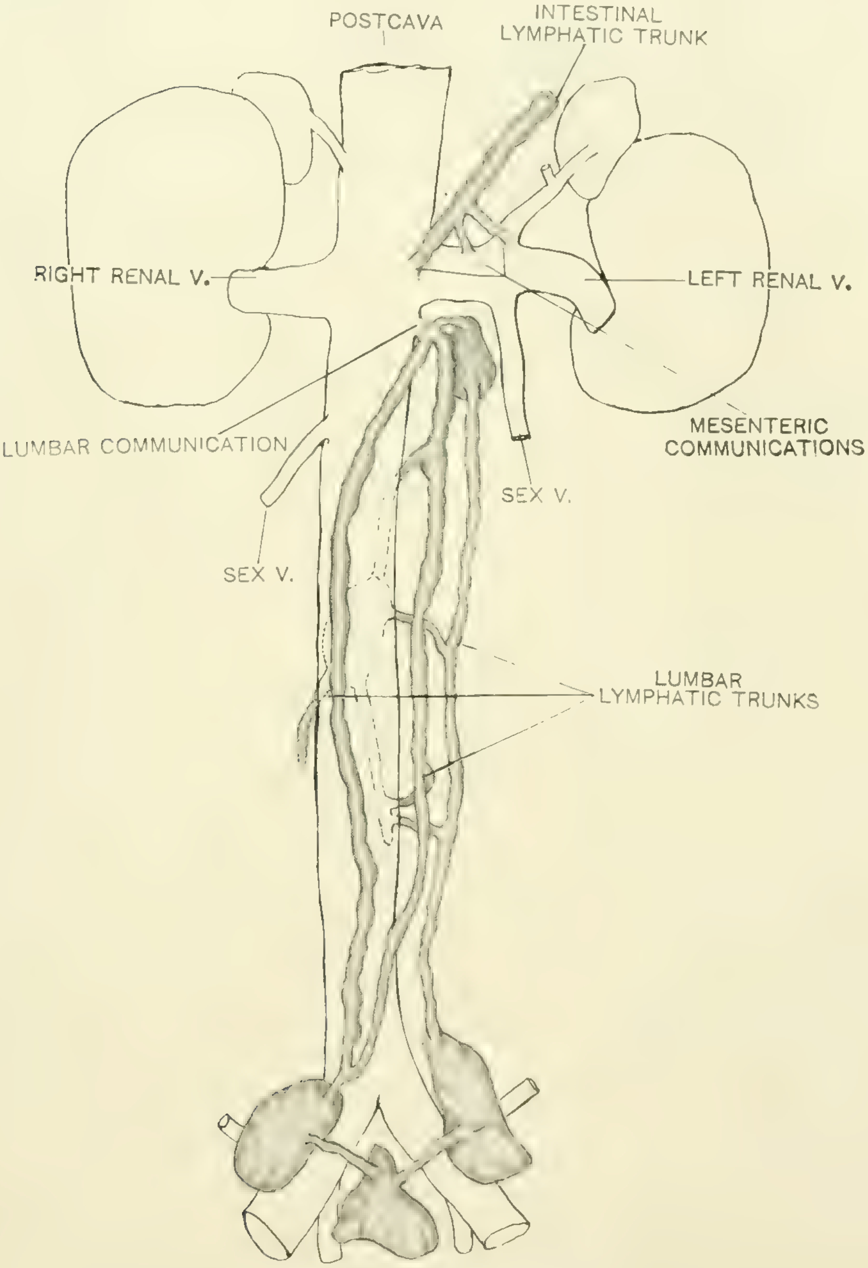


Fig. 10 Midas oedipus, Linn. Pinche Monkey ♂

THE DEVELOPMENT OF THE JUGULAR LYMPH SAC IN BIRDS

A. M. MILLER

From the Anatomical Laboratory of Columbia University

TEN FIGURES

In view of the work done within recent years on the development of the lymphatic system in mammals, it has seemed desirable to extend the study of this system to other vertebrate forms. The investigation of conditions in the chick was undertaken at the suggestion of Dr. Huntington, and has been conducted under his supervision.

The detailed study of closely related early stages in the development of the mammalian jugular lymph sac (Huntington and McClure[1]), and their complete agreement with corresponding genetic stages in reptilian embryos (Huntington[2]) warrant definite conclusions respecting the ontogeny of the avian lymph sac based on a relatively smaller and less closely graded series of embryos. In fact, one of the chief purposes of the publications above referred to is the definite establishment of the genesis of the lymph sac in a representative mammalian form in such detail that it may serve as a comparative basis not only for other mammalian embryos but for embryos of the remaining amniote classes. It is evident that the chick embryos here described and figured could by no possibility agree as completely as they actually do with the corresponding mammalian stages if the development of the jugular lymph sacs differed in any essential respect from that

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[1] George S. Huntington and Charles F. W. McClure: The Anatomy and Development of the Jugular Lymph Sacs in the Domestic Cat (Felis domestica). The American Journal of Anatomy, vol. 10, no. 2, 1910.

[2] George S. Huntington: The Development of the Lymphatic System in the Reptiles. The Anatomical Record, vol. 5, no. 6, 1911.

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now known to obtain in the mammal. The two main purposes of the present communication are therefore:

1. The establishment of the presence of an anterior or jugular lymph sac in avian ontogeny. This will include a brief account of the chief developmental stages, of the formation of a definite sac by coalescence of numerous veno-lymphatic anlagen, its temporary separation from the venous system, and its subsequent secondary and permanent union with that system.
2. The demonstration of the identity of the genesis of the jugular lymph sac in bird and mammal by comparison of corresponding stages in the chick and the cat.

It should be stated here, moreover, that this communication is limited to the specific case of the jugular lymph sacs, and that it does not in any way refer to the development of the systemic lymphatics.

In 1900 Sala[3] published his excellent account of the development of the lymphatics in the chick, but he confined his attention for the most part to the posterior lymph hearts and the thoracic duct. He describes and figures certain vascular channels and mesenchymal 'cords' in the region of the precaval veins, but does not ascribe to these structures any rôle in the formation of lymph sacs (hearts), looking upon them rather as factors in the development of the thoracic duct.

So far as the writer is aware, Mierzejewski[4] is the only investigator who has studied the development of the lymphatics in the cervical region in birds, with the idea that these lymph vessels are derivatives of veins and that they subsequently form structures analogous to the posterior lymph hearts (of Sala). As a matter of fact, Mierzejewski goes only so far as to observe that in the region where the subclavian, vertebral and jugular veins unite, a plexus of lymph vessels (Lymphgefässplexus) arises by enlargement and coalescence of branches of these veins and that

[3] Sala, Luigi: Sviluppo dei cuori linfatici e dei dotti toracici nell' embrione di pollo. Ricerche fatte nel laboratorio de anatomia normale della R. Universita di Roma, vol. 7, 1900.

[4] Mierzejewski, L.: Beitrag zur Entwicklung des Lymphgefässsystems der Vögel (vorläufige Mitteilung). Extrait du bulletin de l'academie des sciences de Cracovie, 1909.

this plexus condenses further to form a 'sponge-like' (schwammartig) structure which is comparable with the posterior lymph heart. He does not state that these venous derivatives break away from the veins and coalesce still further to form a definite sac which subsequently acquires a new connection with the veins.

The present investigation is based upon a study of serial sections of chick embryos and reconstructions made after a slight modification of Born's wax plate method. The embryos were fixed in vom Rath's picro-sublimate-acetic mixture and the sections stained with Delafield's hæmatoxylin and picric acid, or fixed in Zenker's or Bouin's fluid and stained with Weigert's hæmatoxylin and orange G.

Chick embryo of five days and twenty hours. Reconstruction, right side. Fig. 1. The precardinal vein (*1*) pursues a comparatively straight course from the cephalic arch to its junction with the postcardinal (*2*). The postcardinal, after receiving the primitive ulnar vein at the level of the upper limb bud, extends cephalad along the side of the aorta for some distance and then bends ventrad rather abruptly to join the precardinal. The confluence of the two cardinal veins forms the duct of Cuvier (*3*) which bends ventrad and caudad to open into the sinus venosus. The sharp bend in the postcardinal, just caudal to its union with the precardinal, produces a fairly distinct promontory in the cardinal line.

So far as can be determined in this embryo, no intersegmental veins (*4*) open into the precardinal (*1*). The intersegmental veins (*4*) which are distinctly formed empty into the postcardinal (*2*). Four of these which are especially large open upon the dorso-cephalic side of the above mentioned promontory; the others open into the more nearly straight portion of the postcardinal (*2*) at regular intervals as far back as the confluence of the latter vein with the primitive ulnar.

In the tissue dorsal to the straight portion of the precardinal vein (*1*), a collection of vascular islands (*5*) is situated in a manner which indicates the inception of a plexus of vascular channels. In addition to these islands, there are a few short channels which communicate with the precardinal. In the region of and caudal to the promontory there are also several vascular islands (*5*) situated dorso-lateral to the postcardinal (*2*). These islands are farther separated from one another than those along the line of the precardinal. In this region also a few channels open into the postcardinal.

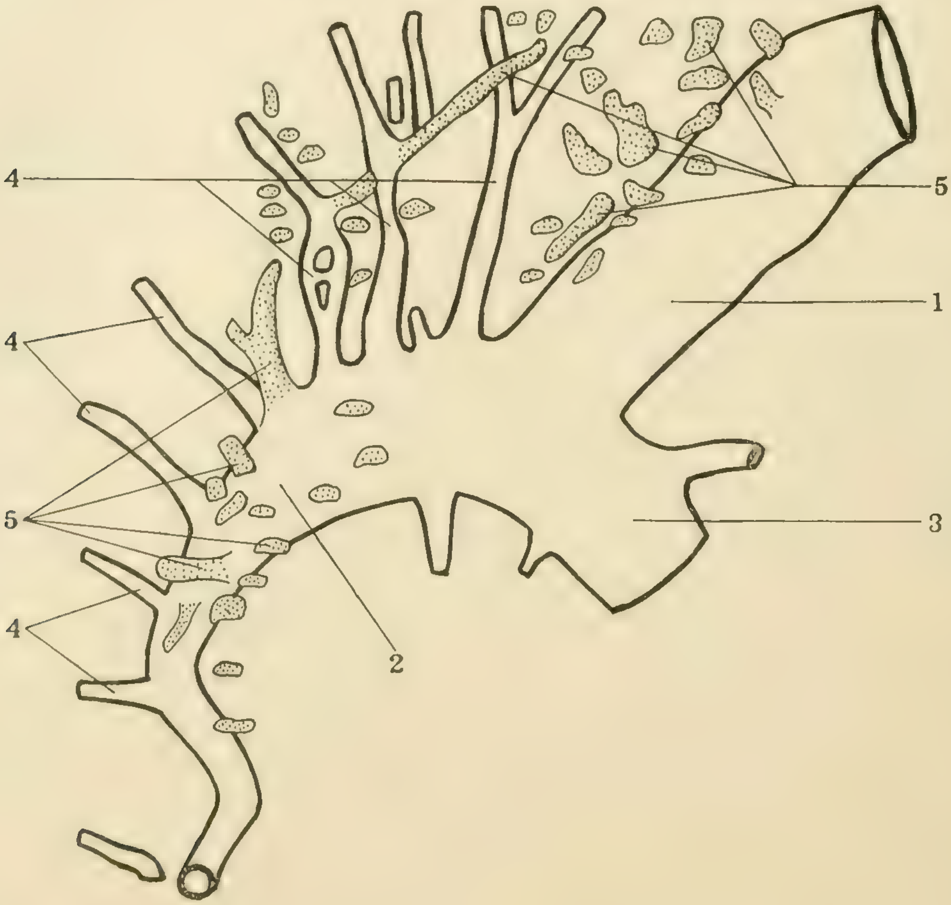

Fig. 1 Diagram drawn from a reconstruction of the veins in the cervical and upper thoracic regions of a chick embryo of five days and twenty hours. Right side. *1*, Precardinal vein; *2*, postcardinal vein; *3*, duct of Cuvier; *4*, intersegmental (dorsal somatic) veins; *5*, lateral group of venous islands (derivatives of and additions to which are represented by stippled areas in succeeding diagrams).

There is a general tendency on the part of the intersegmental veins (*4*) toward a division into medial and lateral branches. The medial branches run close to the aorta and are obviously the dorsal somatic veins proper, being in this region the cervical intersegmental veins (fig. 2, *4*). The lateral branches arise in the same general region in which the above mentioned vascular

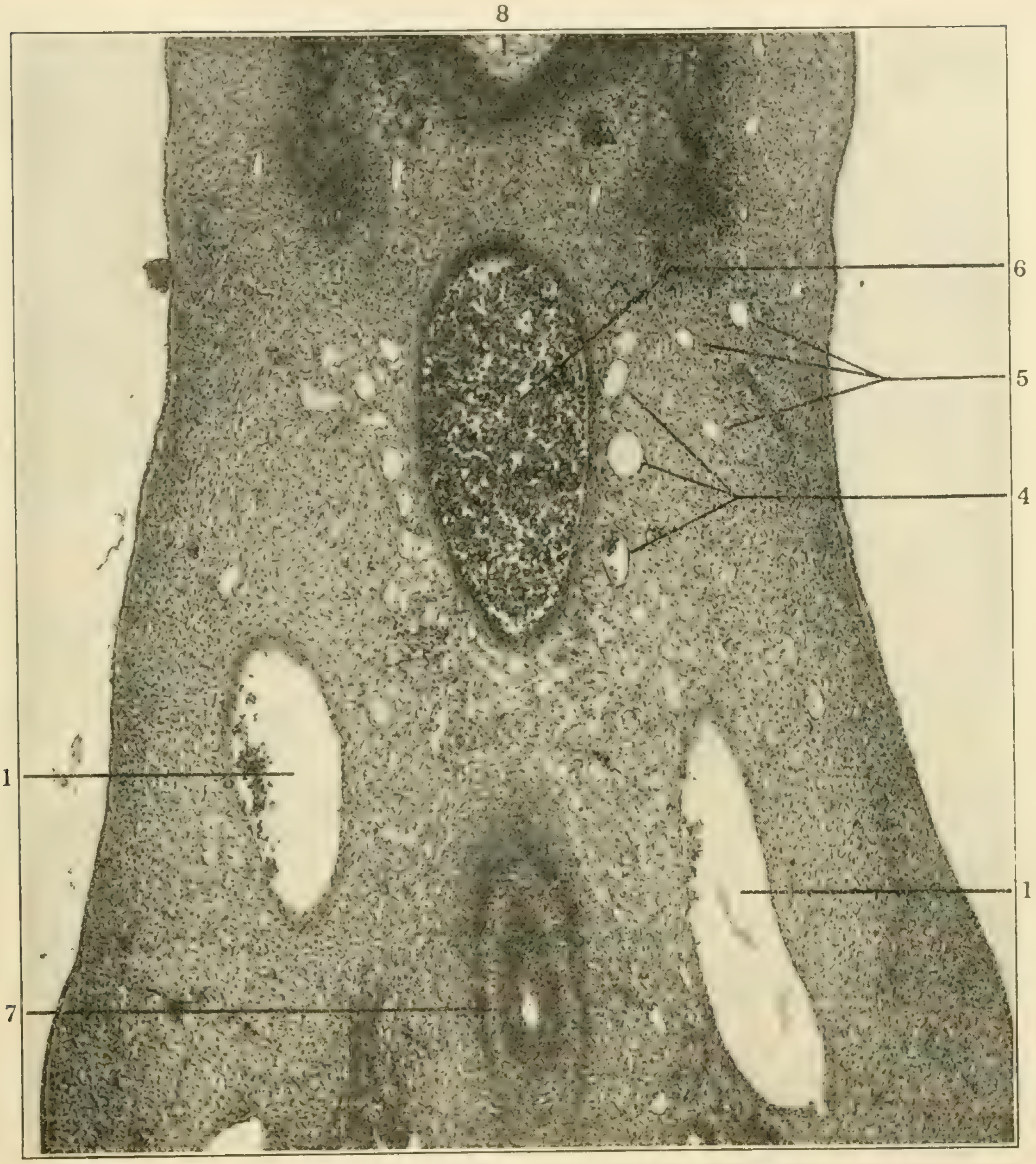

Fig. 2 From a photograph of a section through the cervical region of a chick embryo of five days and twenty hours (embryo from which the reconstruction represented in fig. 1 was made). *1*, Precardinal vein; *4*, intersegmental cervical (dorsal somatic) veins; *5*, lateral group of venous islands (represented in fig. 1 by stippled areas); *6*, aorta; *7*, œsophagus; *8*, notochord.

islands (*5*) are situated. Despite the fact that at this stage there is a tendency toward a separation of the vascular channels and islands into a medial group and a lateral group, they apparently all belong in the same category, namely, an aggregation of vascular channels and islands in the mesenchymal tissue dorsal to the portions of the precardinal and postcardinal veins near their confluence to form the duct of Cuvier. All these channels and islands are obviously venous in character, many of them filled with nucleated red blood cells.

Compared with the mammalian embryo, the conditions in the chick at this stage correspond with those in a cat embryo of 6.2 mm. (Huntington and McClure, figs. 27, 28, 29 and 30). In the chick, however, the vascular islands and channels belonging to the lateral group have not coalesced to such a degree as the corresponding veno-lymphatics in the cat. In the cat the precardinal receives several dorsal tributaries while in the chick, at this stage at least, the dorsal somatic veins proper of this region open into the postcardinal.

Chick embryo of five days and ten hours, 13.5 mm. Reconstruction, right side. Fig. 3. Although this embryo is younger than the preceding, it is farther advanced in development, due probably to different conditions in incubation.

The precardinal vein (*1*) has become elongated both relatively and absolutely, and pursues a moderately curved course from the cephalic arch to the duct of Cuvier (*3*). The proximal portion of the postcardinal (*2*) is relatively smaller than in the preceding stage, and appears rather as a continuation of the primitive ulnar vein than as a part of the original postcardinal. There is no promontory in the cardinal line such as, in the previous stage, was produced by the sharp bend in the proximal end of the postcardinal (compare figs. 1 and 3).

There is in this stage a still more distinct separation of the vascular channels and islands (*5*) dorsal to the cardinal veins (*1, 2*) into a medial and a lateral group than in the stage considered before. Here again the medial group obviously represents the dorsal somatic (cervical intersegmental) veins proper (*4*), while the lateral group (*5*) exhibits a stronger tendency toward a plexiform arrangement.

As in the preceding stage, none of the dorsal somatic veins (*4*) open into the precardinal (*1*). Along the dorso-lateral aspect of the aorta, however, as far back as the level of the duct of Cuvier

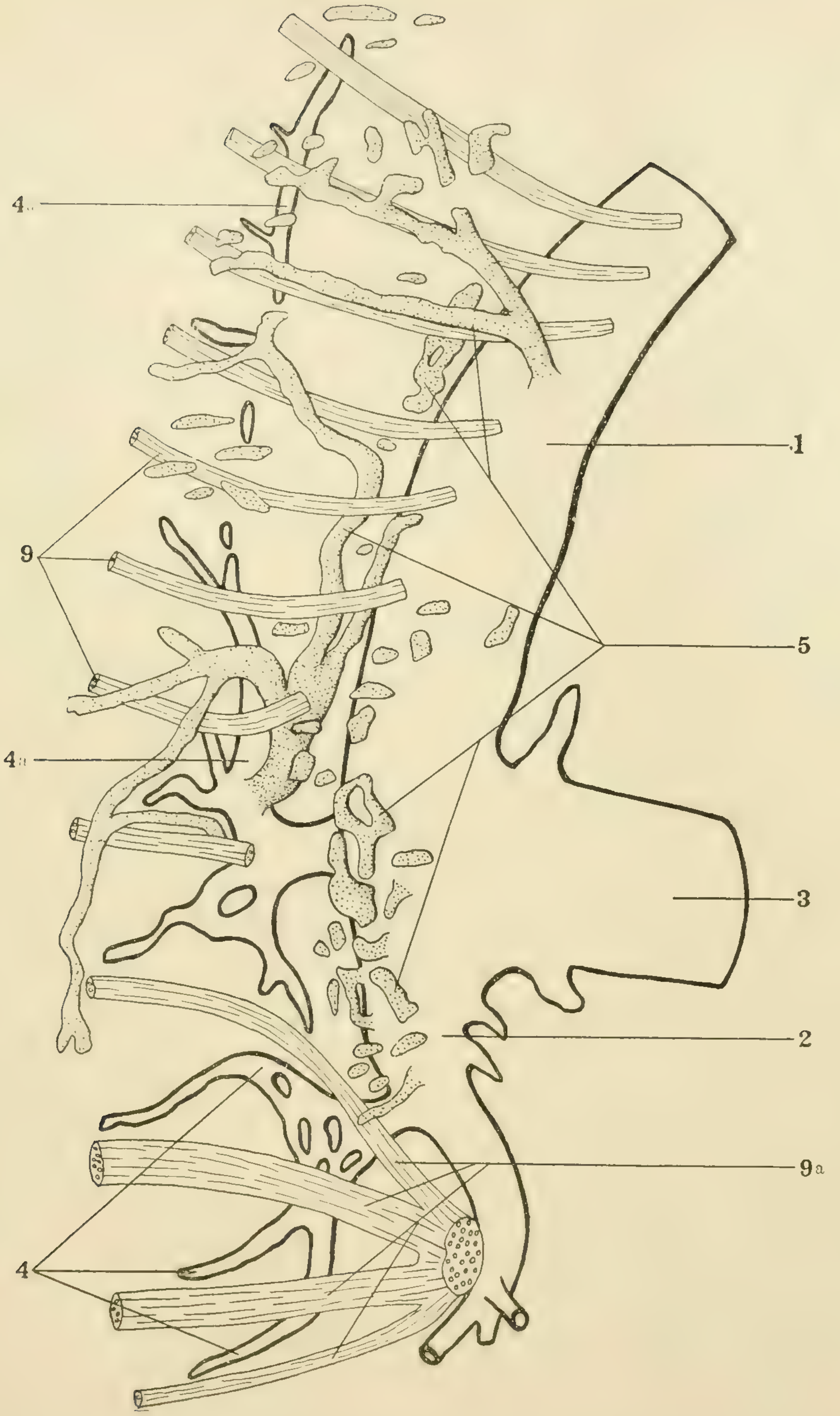

Fig. 3 Diagram drawn from a reconstruction of the veins and nerves in the cervical and upper thoracic regions of a chick embryo of five days and ten hours (13.5 mm.) Right side. *1*, Precardinal vein; *2*, postcardinal vein; *3*, duct of Cuvier; *4*, intersegmental (dorsal somatic) veins; *4a*, vertebral (dorsal somatic) vein; *5*, lateral group of vascular islands and channels—veno-lymphatics; *9*, spinal (cervical) nerves; *9a*, brachial plexus.

(*3*), there is a venous plexus that receives dorsal branches corresponding to the segmental nerves (*9*). This plexus shows the incipient stages of the formation of a longitudinal trunk, and in a few places sends anastomotic branches across the medial line to a corresponding plexus on the opposite side. At the level of the duct of Cuvier (*3*), and lateral to the aorta, there is a very distinct plexus of veins which receives intersegmental branches from the region of the vertebral anlagen, and which converges ventrally to form a discrete and fairly large vessel. This vessel opens into the dorsal side of the cardinal directly opposite the duct of Cuvier (*3*). The more cephalic venous channels are confluent with those situated at the level of the Cuvierian duct, so that if a longitudinal trunk is formed out of the former it will obviously join the above mentioned vessel that leads into the cardinal, and will constitute the longitudinal vertebral vein (*4a*). At the level of the brachial plexus of nerves (*9a*), the dorsal somatic veins (*4*) open separately into the postcardinal (*2*) which is now broken up into a number of smaller channels in the mesonephros.

The lateral group of vascular islands and channels (*5*) is distinguished from that of the preceding stage by two main features. In the first place many of the islands have coalesced to form channels of considerable length. In the region of the precardinal these channels show a decidedly segmental arrangement. At the level of the duct of Cuvier (*3*) and thence for a short distance caudad, they are characterized by a more irregular plexiform arrangement. In the second place the vascular channels in question communicate more freely with the cardinal veins (*1*, *2*). There is one very distinct vessel, representing a convergence of the more cephalic channels, that opens into the lateral side of the precardinal (*1*). Another vessel, which arises through the convergence of a number of the channels dorsal to the duct of Cuvier, opens into the trunk formed by the converging dorsal somatic plexus.

The majority of the segmentally arranged vessels of the lateral group (*5*) receive dorsal branches from the mesenchymal tissue between the vertebral anlagen and the epidermis. At the level of the duct of Cuvier (*3*), two or three of these prolongations have anastomosed to form a longitudinal channel situated lateral to the vertebral anlagen. As a final consideration of this stage, a view of the lateral group of channels, especially those connected with or situated near the precardinal vein, gives the impression that they are leading toward the formation of a longitudinally arranged plexus, which will be situated dorsal to the cardinal line. These channels are obviously of venous origin and are undoubtedly homologous with the veno-lymphatic vessels in the mammalian embryo as exemplified in a cat embryo of 7 mm. (Huntington and McClure, figs. 33, 34, 35, 36).

Chick embryo of six days. Reconstruction, right side. Fig. 4. The medial or dorsal somatic group of vessels is not represented in fig. 4. The changes between the preceding stage of development (chick embryo of five days and ten hours) and the one under consideration here have affected principally the lateral group of vascular elements (*5*). The medial and lateral groups have now become completely separated and are absolutely independent of each other. Whatever communication existed in the 5-day-and-10-hour stage has been wholly dissolved, the medial group now constituting the dorsal somatic vessels exclusively, the lateral group (*5*) forming a large and complicated plexus of channels which calls for further observation and interpretation.

The vascular channels which, in the preceding stage (fig. 3, *5*), occupied a position dorsal to the proximal ends of the precardinal and postcardinal veins, have enlarged into an enormously complicated plexus (fig. 4, *5*) which no longer possesses the former fairly distinct segmental character, has become entirely separated from the dorso-medial somatic tributaries, and has further lost the previous rather free communication with the cardinal veins, being now entirely detached from the main venous trunks with the exception of a single small anterior capillary connection. The plexus is relatively shorter than that of the previous stage, being confined for the most part to the region of the duct of Cuvier (*3*) and the proximal end of the precardinal (*1*) and extending but a short distance along the postcardinal line (*2*). It also extends farther around on the lateral aspect of the precardinal. The component channels have a general longitudinal trend, and vary in size from the smallest capillary to vessels as large as or larger than the precardinal vein (fig. 4, *5* and fig. 5, *5*). For the most part the vessels are intercommunicating, but a few islands lie in the peripheral region. The walls of the channels are composed merely of endothelium, without visible condensation of mesenchyme around them (fig. 5, *5*). A great majority of the channels are empty; a few situated peripherally in the group, contain small numbers of nucleated red blood cells.

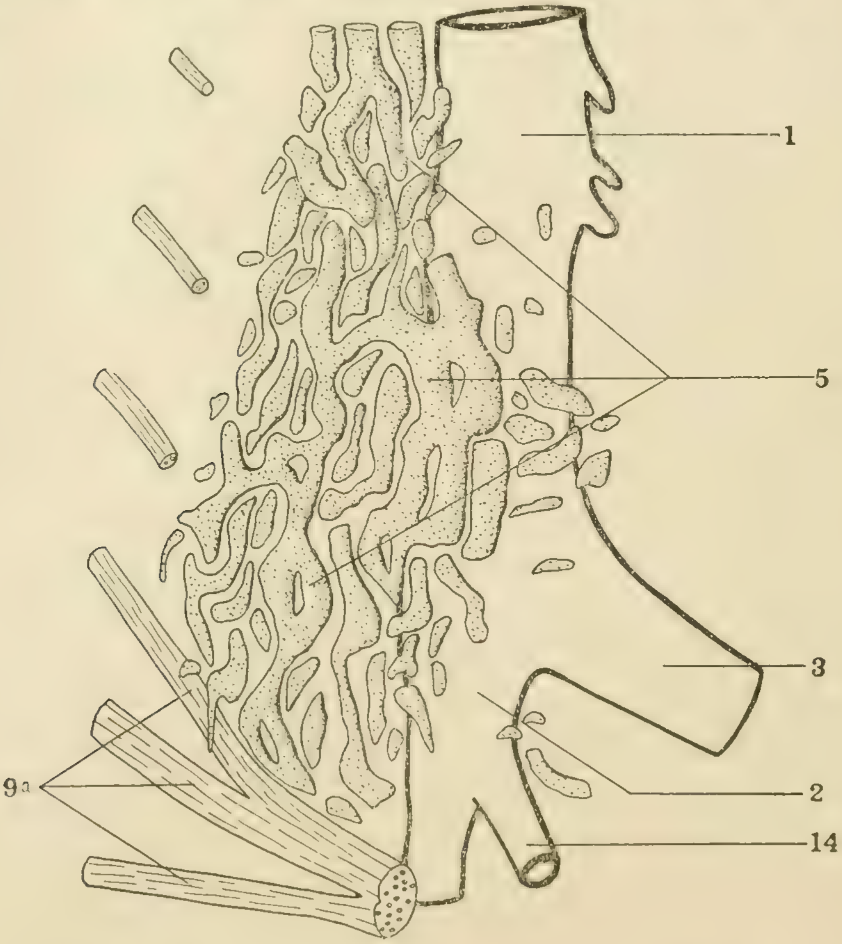

Fig. 4 Diagram drawn from a reconstruction of the veins, veno-lymphatics (prelymphatics) and nerves in the cervical and upper thoracic regions of a chick embryo of six days. Right side. *1*, Precardinal vein; *2*, postcardinal vein; *3*, duct of Cuvier; *5*, veno-lymphatic (prelymphatic) plexus, the forerunner of the jugular lymph sac; *9a*, brachial plexus; *14*, subclavian vein.

The conditions met with in this stage resemble very closely the conditions in a 10 mm. cat embryo (Huntington and McClure, fig. 49), with the noteworthy exception that in the chick the plexus has already lost its connection with the cardinal veins.

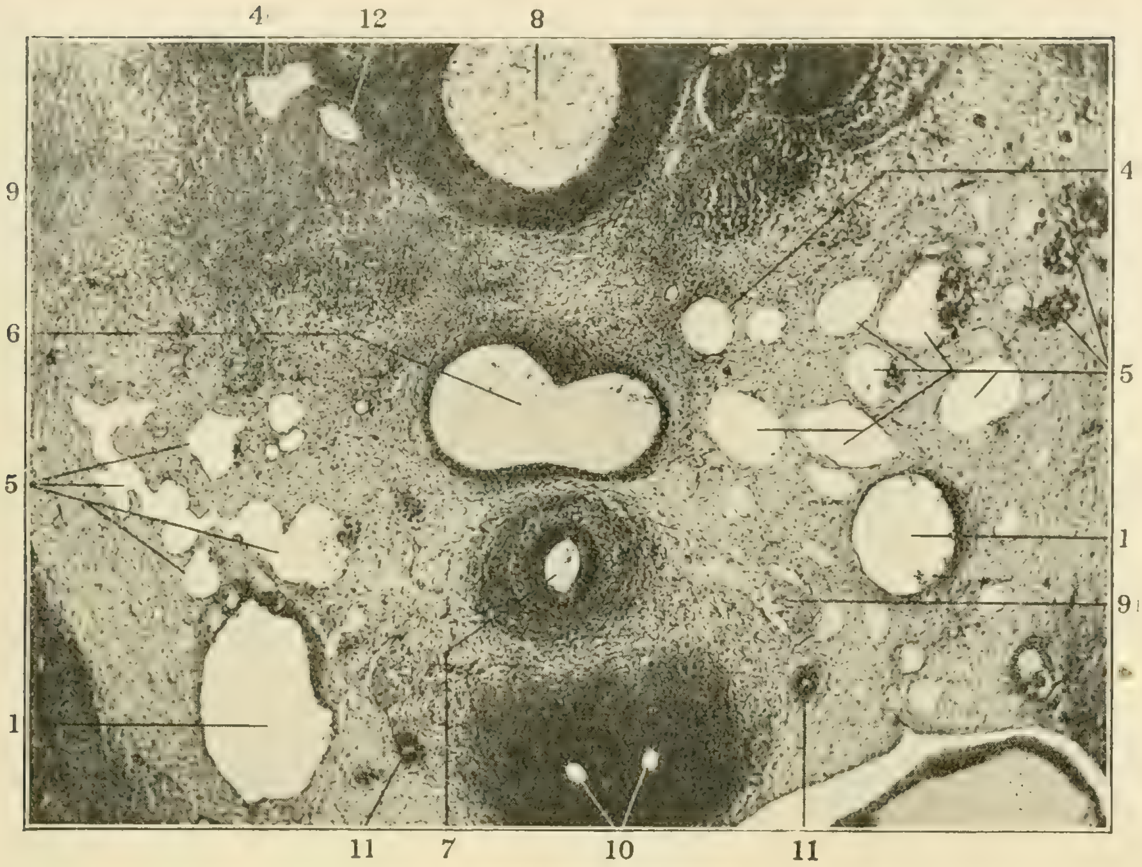

Fig. 5 From a photograph of a transvere section through the upper thoracic region of a chick embryo of six days. *1*, Precardinal vein; *4a*, vertebral vein; *4b*, branch of vertebral vein; *5*, veno-lymphatic (prelymphatic) plexus; *6*, aorta; *7*, œsophagus; *8*, notochord; *9*, spinal nerve; *9b*, vagus nerve; *10*, bronchi; *11*, pulmonary artery; *12*, vertebral artery.

This relatively earlier loss of communication between the veno-lymphatics and the venous system in the chick is probably due to different rates of metabolism in the two classes of animals. Following the dissolution of the connection between veno-lymphatics and veins, the plexus of channels in question may now be termed prelymphatics, in accordance with the usage of Huntington and McClure.

The evolution of the complicated and extensive veno-lymphatic plexus of the six day chick (figs. 4 and 5) from the simpler dorso-lateral group of venous channels and islands of the 5-day-and-10-hour embryo (fig. 3) is shown to occur in intervening stages by progressive growth in size and number of the vascular elements involved. The dorso-lateral venous channels of the earlier stages (fig. 3) rapidly increase, assume a more plexiform character, successively amalgamate with one another, and become separated from the permanent veins, until the conditions shown in figs. 4 and 5 are attained.

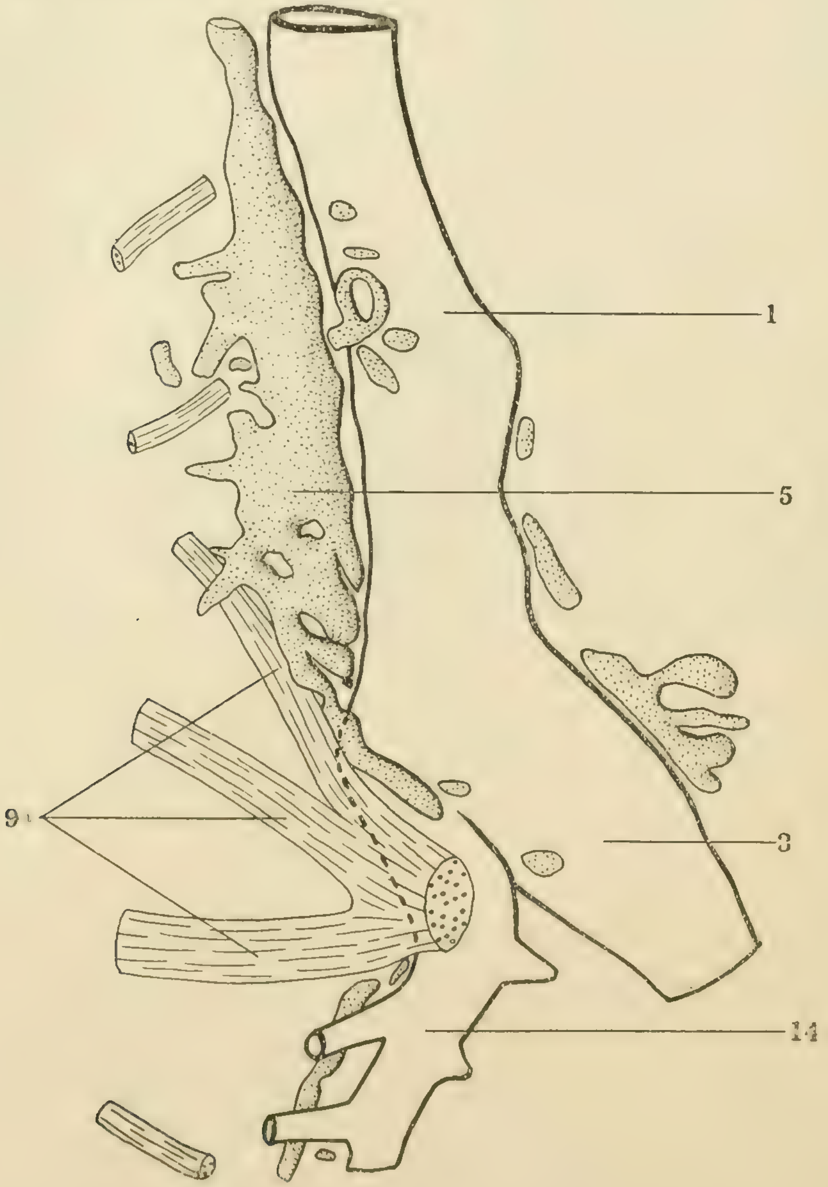

Fig. 6 Diagram drawn from a reconstruction of the veins, prelymphatic sac and nerves in the upper thoracic region of a chick embryo of seven days. Right side. *1*, Precardinal vein; *3*, duct of Cuvier; *5*, prelymphatic sac; *9a*, brachial plexus; *14*, subclavian vein.

Chick embryo of seven days. Reconstruction, right side. Fig. 6. Out of the complicated plexus of channels present in the six day embryo (fig. 4, *5*) there has arisen a quite definite sac-like structure, which extends cephalad from the level of the duct of Cuvier along the dorsal aspect of the precardinal vein, and is almost equal in caliber to the vein itself (fig. 6, *5*). This structure, which is the immediate forerunner of the jugular lymph sac, although it does not at this stage communicate with the venous system, is the result of the enlargement and coalescence of the numerous smaller channels of the preceding stages. While the sac as a whole is relatively straight, its wall is exceedingly irregular. This irregularity is due to diverticula which represent channels not yet completely incorporated in the sac. A few isolated channels also are situated in the immediate vicinity of the sac.

As noted above, the sac at this stage does not in any way communicate with the venous system. Consequently it is properly spoken of as a prelymphatic. It comprises an endothelial bag which lies free in the mesenchyme, and is practically destitute of blood cells, a few being present in some of the diverticula (fig. 7, *5*).

There can be no doubt that the veno-lymphatic forerunner of the jugular lymph sac loses connection with the venous system, and remains free for a considerable period. This disconnection is shown to be absolute in the seven day chick, where the wall of the prelymphatic sac and the wall of the vein do not even approximate (fig. 6, *5* and fig. 7, *5*). In the six day chick the plexiform group of channels is free from the vein except, as already noted, for one capillary communication near the cephalic end. Examination of intermediate stages, from which no reconstructions were made, also showed the lack of communication. The first instance of a new connection was observed in an embryo of eight days and fourteen hours. This temporary disconnection between the anlage of the lymph sac and the veins in the chick coincides with the similar condition in the mammal. For example, in a cat embryo of 10.7 mm. (Huntington and McClure, fig. 59, also page 294), to which the stage in the chick under consideration corresponds most closely, the empty prelymphatic sac is not in communication with the venous system. Observation thus far, however, goes to show that the disconnected condition is probably of longer duration in the chick than in the mammal.

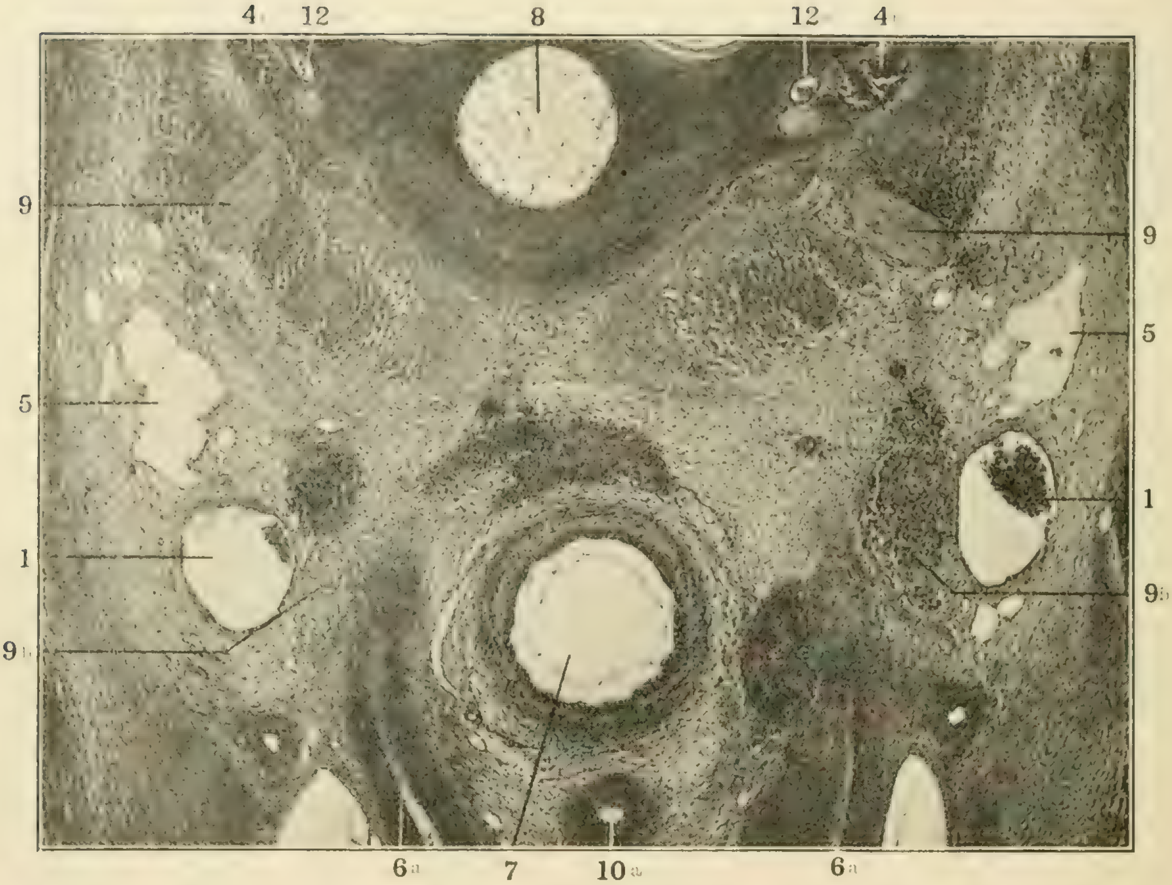

Fig. 7 From a photograph of a transverse section through the upper thoracic region of a chick embryo of seven days. *1,* Precardinal vein; *4b,* branch of vertebral vein; *5,* prelymphatic sac; *6a,* aortic arch; *7,* œsophagus; *8,* notochord; *9,* spinal nerves; *9b,* vagus nerve, with the ganglion nodosum on the right; *10a,* trachea; *12a,* branch of vertebral artery.

Chick embryo of eight days and fourteen hours. Reconstruction, right side. Fig. 8. Passing on to a considerably older embryo (eight days and fourteen hours), we find the lymph sac apparently at the height of its development. It consists here of an elongated bag (*5*) extending some distance cephalad from the level of the subclavian vein (*14*). Its caliber is approximately equal to that of the jugular (precardinal) vein (*1*). Situated ventral to the brachial plexus of nerves (*9a*), which indents its dorsal wall, it lies in close apposition to the dorsal surface of the jugular vein. The ganglion nodosum of the vagus nerve indents its mesial wall. Its length is about 1.5 mm. and its greatest diameter about 0.5 mm.[5]

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[5] These measurements were computed from the number and thickness of the sections involving the lymph sac.

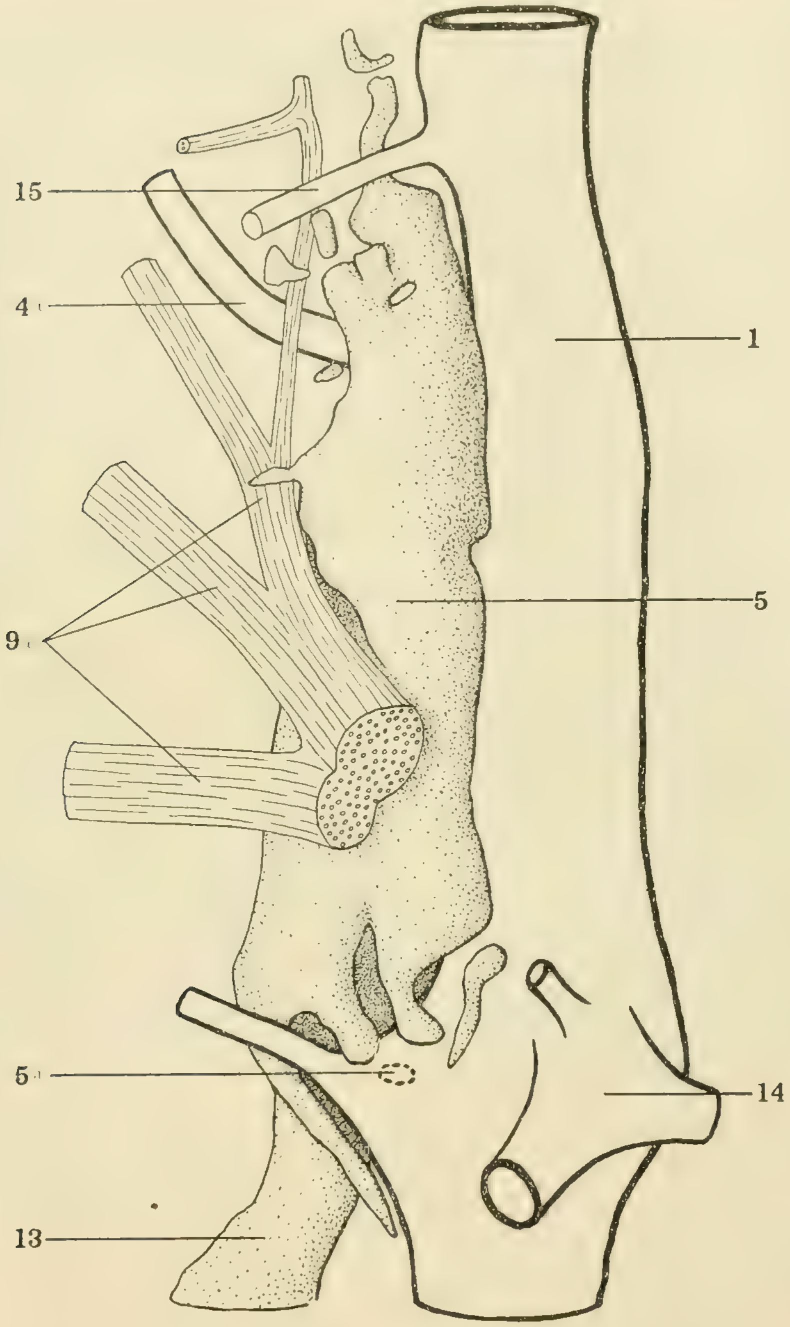

Fig. 8 Diagram drawn from a reconstruction of the veins, jugular lymph sac and nerves in a chick embryo of eight days and fourteen hours. Right side. *1*, Precardinal vein; *4a*, vertebral vein; *5*, jugular lymph sac; *5a*, the dotted oval shows the position of the tap of the jugular lymph sac (see figs. 9 and 10); *9a*, brachial plexus; *13*, thoracic duct; *14*, subclavian vein; *15*, subcutaneous lymphatic duct.

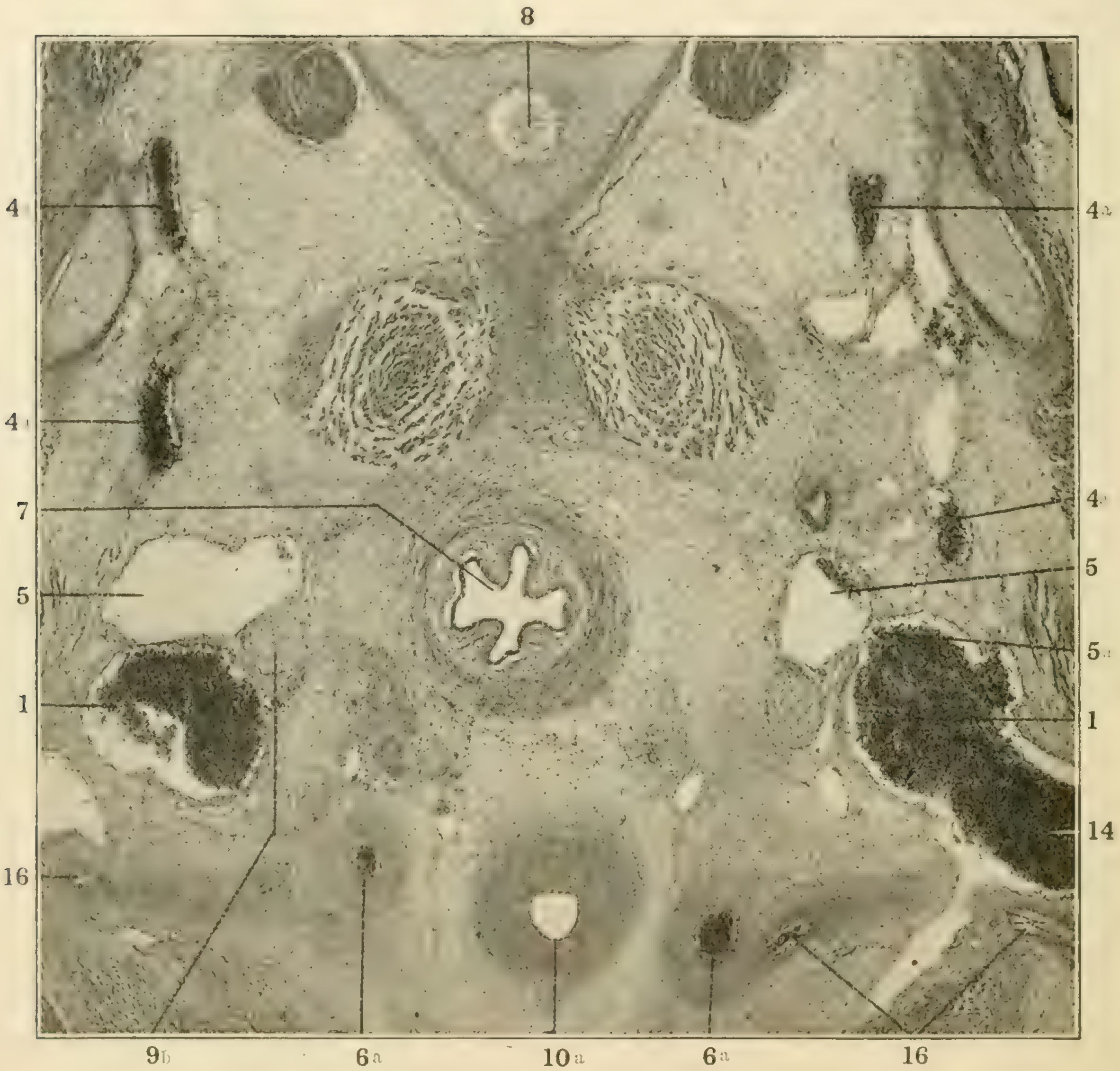

Fig. 9 From a photograph of a transverse section through the upper thoracic region of a chick embryo of eight days and fourteen hours. *1*, Precardinal vein; *4a*, vertebral vein; *5*, jugular lymph sac with tap at *5a* on the right; *6a*, common carotid artery; *7*, œsophagus; *8*, notochord; *9b*, vagus nerve; *10a*, trachea; *14*, subclavian vein; *16*, subclavian artery.

The sac at this stage, having acquired a secondary and new connection with the venous system, may be properly spoken of as the lymph sac. The new communication with the jugular (precardinal) vein (*1*) is established on the dorso-mesial aspect of the vein about opposite the opening of the subclavian (fig. 8, *5a* and fig. 9, *5a*). The conditions in this particular stage indicate that the union has taken place merely through apposition of the wall of the sac with the wall of the vein and a subsequent dissolution of the contiguous portions of the two walls. There is no indication of valve formation at the tap, as has been shown to be the case in the mammal. The absence of valves guarding the opening between the lymph sac and the vein may be due to the fact that the tap had just been established in the 8-day-and-14-hour chick. Valve formation may be a subsequent process, as it actually is in the mammal, but further examination of later stages will be necessary to settle this point. The nature of the communication or tap at this stage is shown in fig. 9, *5a*, on the right side, and in fig. 10, *5a*; its position is shown in fig. 8 by the dotted oval *5a*.

The wall of the sac is composed of endothelium and a thin layer of mesenchyme (fig. 10). No smooth muscle cells can be determined in this layer, although that may be due to the fact that they are insufficiently differentiated by this method of staining. Mierzejewski states that he has observed rhythmical contractions of the anterior lymph heart or sac in embryos of eight days.

At its caudal end the lymph sac is joined by the thoracic duct (fig. 8, *13*). The above mentioned tap, from its position, is in all probability homologous with the jugulo-subclavian tap in the mammal (cf. fig. 62, 16 mm. cat embryo, Huntington and Mc-McClure). In the chick at this stage a second tap is lacking, but it is not improbable that a study of later stages will reveal a homologue of the common jugular tap in the mammal. According to the descriptions and figures of the anatomy of the lymphatics in birds, a branch of the lymphatic trunk from the head and neck, which occurs as a variant on the right side, opens into the jugular or the precaval vein. There is good reason to assume that there exists a considerable variability in the communications between the lymphatic trunks and the veins, as in the cat, and that a careful investigation of the anatomical relations in the adult bird will serve to bring them into accord with the conditions in the mammal.

A lymph sac in the adult bird has not been described or figured, so far as the writer is aware. However, the conditions in the

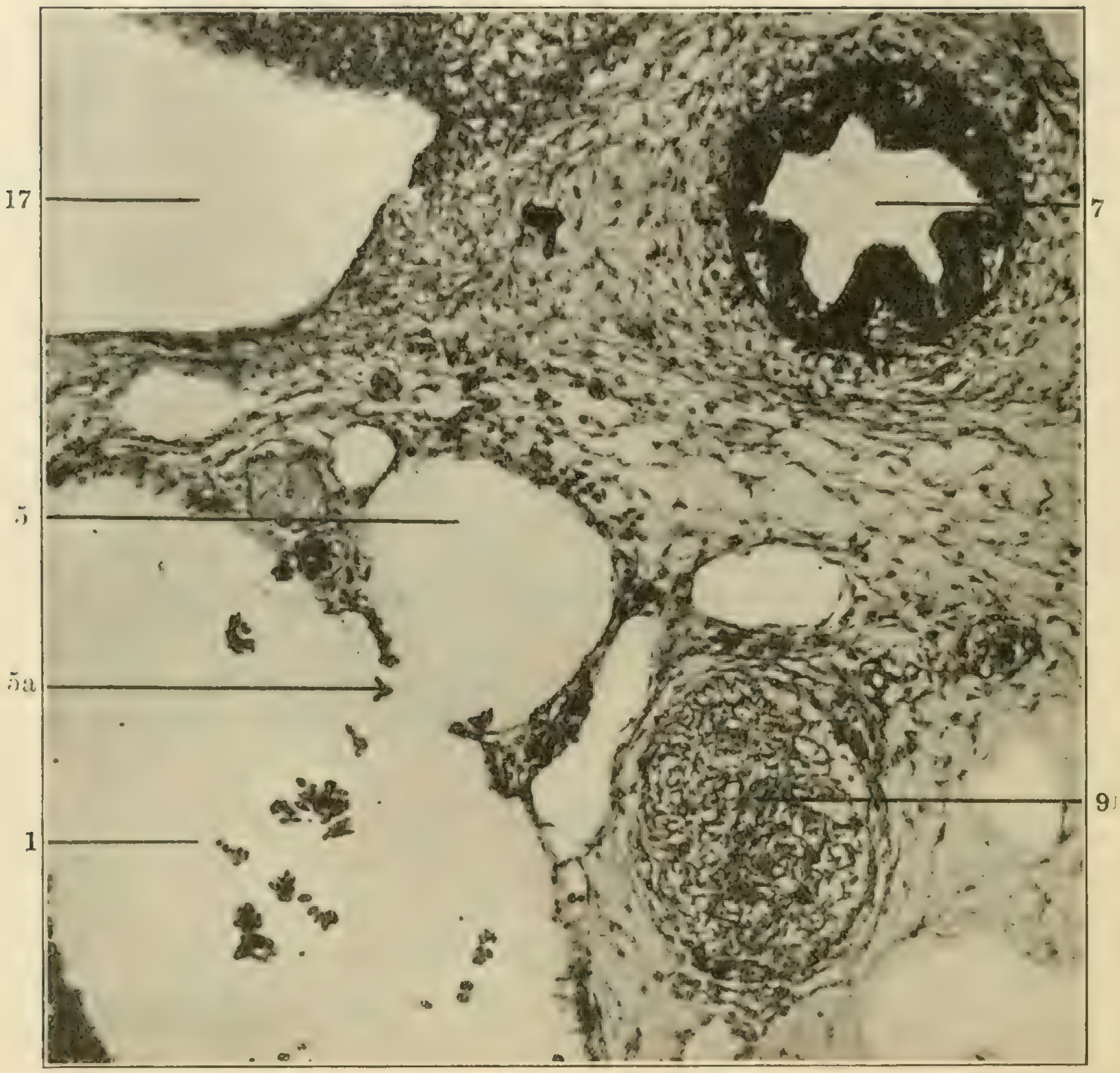

Fig. 10 From a photograph of a transverse section through the thoracic region of a chick embryo of eight days and fourteen hours. *1*, Precardinal vein; *5*, jugular lymph sac with tap, at *5a*, on the dorso-mesial side of the precardinal vein; *7*, œsophagus; *9b*, vagus nerve; *17*, pleural cavity.

chick embryos show that it develops in this form in exact agreement with the corresponding mammalian ontogenetic stages. It is therefore probable that careful examination of the anterior lymphatico-venous connections of the adult bird will demonstrate the persistence of the sac in a modified form, as has been shown to be the case in the mammal.

SUMMARY

Venous islands and channels appear in the mesenchymal tissue dorsolateral to the precardinal and postcardinal veins near their junction to form the duct of Cuvier. These islands and channels constitute what has been called in this article a lateral group of vascular elements in contradistinction to a mesial group which forms the dorsal somatic (intersegmental) veins, although both groups are at first rather closely associated. Some of the lateral elements open into the cardinal veins (figs. 1, 2 and 3). The elements of the lateral group enlarge and new capillaries are added until, eventually, the group as a whole constitutes an extensive plexus of veno-lymphatic channels, the plexus also becoming distinctly separated from the more mesially situated dorsal somatic veins (figs. 4 and 5). The veno-lymphatics, by progressive enlargement and coalescence, then develop into a distinct sac-like structure and in the meantime become completely cut off from the venous system, thus forming the prelymphatic sac (figs. 6 and 7). The prelymphatic sac subsequently acquires secondary communication with the venous system through a tap situated on the dorsomesial side of the precardinal (jugular) vein at the level of the subclavian, and thus becomes the jugular lymph sac (figs. 8, 9 and 10).

THE FIFTH AORTIC ARCH OF MAMMALIAN EMBRYOS; THE NATURE OF THE LAST PHARYNGEAL EVAGINATION

FRANK REAGAN

Contribution Number 121 from the Zoölogical Laboratory of Indiana University

SIXTEEN FIGURES

PART 1

The question of the existence of six aortic arches in mammalian embryos has recently received much attention. In the minds of some, perhaps, it is definitely and satisfactorily settled; yet the known facts and the conclusions drawn from them are diverse and conflicting. For this reason, anything further to be added may be of interest.

The significance of the question is well understood. A demonstration of six arches would serve to bring into line the branchial circulation of mammals with that of the lower vertebrates, and to strengthen the belief in the similarity of ontogenies in related groups. By Lehmann and Locy the view has been advanced, that, upon a demonstration of the same number of arches in all air-breathing groups depends the comparability of the arches giving rise to the pulmonary arteries. This view seems altogether unnecessary since, if it be granted that mammals have only five pairs of arches, all doubt of comparability should fall on the more transitory and questionable vessels. To deny the similarity of the pulmonic arches because of a variable number of arches anterior to them, is as illogical as to doubt the identity of the systemic arches because of the fact that different numbers of arches may exist posterior to them. Because of the constancy of their characteristics, the identity of the pulmonic arches of all air-breathing vertebrates is well established.

All workers are agreed that the transitory vessel representing a new arch is to be looked for between the fourth and pulmonic arches.

Although the results of previous observers have been rather exhaustively discussed, mention may profitably be made of points which have direct bearing on the present work.

The first evidence in favor of the predicted existence of a new arch in mammals, was adduced by Zimmermann ('89), when he found in a 7 mm. human embryo, a small vessel arising from and re-entering the posterior side of the fourth arch. He also described a complete and typical fifth arch in an eleven day rabbit. The arch arose from the ventral aorta and emptied into the aortic root, not far from the dorsal lumen of the pulmonic arch.

Tandler ('02) found, in a rat embryo, a broad connection between the fourth and pulmonic arches, parallel to the dorsal aorta; he identified it as a fifth arch, despite the fact that it was without connection with either aorta, and that there was no corresponding pharyngeal pouch. In two human embryos he found vessels connecting the ventral aortae with dorsal portions of the pulmonic arches.

Lehmann ('05) has figured a complete arch for the pig, and has described it as follows:

> A short distance from its union with the truncus arteriosus the fourth arch increases greatly in width and there is given off from its posterior side near the middle of the arch, a smaller, but perfectly distinct vessel which, bending slightly downwards, follows along the course of the fourth arch and joins the dorsal aorta immediately beneath it. Just ventral to its union with the aortic root there passes back from the rudimentary vessel, a branch which joins the sixth arch immediately ventral to its union with the dorsal aorta.

From this description we are to interpret the portion of the vessel joining the aorta as the distal portion of the arch proper, and the vessel to the pulmonic as a branch of it. These indeed would be conditions requisite to a typical arch; but Locy's interpretation of the same vessel is not in accord with the above view when he describes it as "passing dorsad and caudad to unite with the pulmonic arch near the union of the latter with the aortic root." He also states that "it is connected with the aortic root by an independent branch." This conclusion he bases on the results of previous observers and upon the other figures of Lehmann, but from her fig. 10, it must be admitted that there is as much and perhaps more ground for Lehmann's interpretation, because the blind spurs on the aortic root in that figure are more prominent than those on the pulmonic vessel.

Lewis ('06) having investigated the conditions in the rabbit and the pig, maintained that the vascular irregularities, blind spurs, and anastomosing sinuses near the bases of the fourth and pulmonic arches furnish no evidence that a typical arch exists between them. His figures afford strong evidence to support this view. He regards the attempts to demonstrate a fifth arch as "morphological speculations of extreme interest" but adds his belief that "the general recognition of a new arch in mammals seems to be due to those considerations which led Boas to predict it rather than those which come from the study of mammalian embryos themselves."

In mole embryos of 4.7, 5, 5.5, and 6 mm., Soulié and Bonne ('08) found a number of complete vessels arising from both the truncus anteriosus and the fourth arch. Their 5.5 mm. embryo is of interest becaue of a more typical fifth vessel. This vessel is described as "entierement distinct et isolé" from the fourth and sixth arches. Its origin from the aortic bulb is given as 30μ above the ventral lumen of the fourth arch in a plane vertical to it. (From this description the ventral aorta is considered as extending dorsally and laterally to the point of origin of the fifth arch and including the common trunk of the fourth and fifth arches.) On leaving the bulbus it turns outwards, curves, and applies itself against the external face of the fifth pouch, embracing the latter in its concavity. On leaving the fifth visceral arch it turns freely to unite with the aortic root, about 80μ from the lumen of the pulmonic arch. Their 6 mm. embryo 'C' shows remarkably distinct fifth visceral arches. They maintain themselves to have demonstrated perfect fifth aortic arches (regarding those connected with the pulmonic as typical), but admit that the work of other observers may profitably be annexed to their own.

In a dicephalous lamb, three or four weeks after its birth, a vessel suggesting a fifth arch was found by Bishop ('08). It was seen as a slender sinus, connecting the two component aortic trunks. Since the more stable arches, as such, had either degenerated or become greatly modified, and since the vessel had no connection with either aortic root, it affords rather uncertain evidence of a fifth arch.

In cat embryos, Coulter ('09) found spurs and sinuses which he suggests as rudiments of a fifth vessel, but in no case did he find a complete arch.

If we are to be so exacting as to demand of this new arch, those qualifications and characteristics common to other arches with which we attempt to prove the vessel homologous, it will be seen that the only typical fifth arches yet demonstrated, are those of the eleven day rabbit of Zimmermann, and of the 5.5 mm. mole of Soulié and Bonne. These are described as typical in that they were said to connect the aortae and occupied distinct visceral arches. But the description of the vessel in the Zimmermann embryo was unaccompanied by figures; furthermore, the existence of the vessel in the rabbit has been doubted by Lewis (assent to Lewis's view has been given by Coulter). Also, from the description of the 5.5 mm. mole of Soulié and Bonne, the ventral aorta is considered as extending to a point 30μ vertically above the lumen of the fourth arch, so that the common trunk of the fourth and fifth arches must have been interpreted as ventral aorta. This interpretation is allowable, yet an arch arising more ventrally would be more typical. From their reconstruction 'A,' the dorsal portions of the fifth vessel and the pulmonic arch seem sufficiently intimate to justify their being regarded as entering the dorsal aorta in common. The supposition is in harmony with the view of Coulter in which he, reviewing the results of Soulié and Bonne, stated that their fifth arches emptied "in every case into the dorsal aorta in common with the pulmonic arch." At any rate, this intimacy renders the arch less typical. From the foregoing consideration, a demonstration of an arch more typical than those just mentioned seems desirable, even though it be granted with Soulié and Bonne that they may have successfully demonstrated their "phylogenetic souvenir."

Since the extreme irregularities back of the systemic arch render the existence of a typical "fifth" arch more doubtful, the following question naturally suggests itself: do such irregularities occur in connection with the other arches; if so to what extent; and is a tendency towards bi-lateral duplication of irregularities more strongly displayed in the region of the new vessel than elsewhere?

In order to determine these points and to throw as much light as possible on the probable nature of this fifth vessel and its associated pharyngeal parts, a study was made of one hundred and fifty pig embryos,[1] between stages of eighteen somites and 15 mm. Most of the embryos studied were between and including the stages of 8.5 and 9.5 mm.

In embryos smaller than 6 mm., marked irregularities were rare. The first, second and third arches often showed blunt protuberances and rough walls. These seemed more common on each arch at the height of its development.

In an embryo of 6.5 mm., a vessel of about one third the calibre of the fourth arch (fig. 15) was found springing from the middle of the anterior side of that arch, and returning to it near its dorsal lumen. This smaller vessel is a trifle similar to that of the human embryo of Zimmermann, except that it is found on the anterior face of the fourth arch. The conditions in fig. 15 would indicate that the vessel described by Zimmermann was merely a division of the fourth arch.

At the stage of 7 mm., short spurs were occasionally present near the ventral end of the fourth arch, projecting caudally. Short projections were common on all portions of the posterior face of the fourth arch. Stages of 8 mm. showed slightly more irregularities in the region of the fifth vessel than in the preceding stage. In a few 8.5 mm. embryos, slender dorsal connections between the fourth and pulmonic arches were observed. Projecting into the dorsal aorta and sometimes piercing it, 'islands' of mesoderm were often found. There was a slight tendency towards a duplication of these conditions on opposite sides.

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[1] I am much indebted to Professors C. H. Eigenmann and F. Payne for direction and helpful criticism of the work undertaken. Acknowledgment of indebtedness is also due Professor Frank R. Lillie for permission to consult the embryological collection of the University of Chicago, and Professor C. H. Spurgeon of Drury College, for the use of his excellent private collection. Besides the embryos of these collections, those of the embryological cabinet of Indiana University, a number of series of my own preparation, many student preparations were also examined.

An 8.6 mm. pig (measured after having been killed in Zenker's fluid and dehydrated), number 78.S of the C. H. Spurgeon collection, is of unusual interest because of the remarkably well developed fifth arch. The vessel exists typically on the right side without dorsal connection with either of the neighboring arches.

Figs. 6 and 7 are from wax reconstructions of the branchial circulation of the embryo. They were made by the method of Born. The cavities of the arches are represented as solid. The entodern of the pharynx and the ectodern of a portion of the body wall are represented as such. In maintaining perspective, some parts of the model may appear to have been drawn out of proportion, but apparent discrepancies will be accounted for by a comparison of figures. In fig. 7, for instance, the intimacy of the fifth vessel with the ventral aorta would seem exaggerated, since it is impossible to show the extent of the ventral aorta in a lateral view. On the other hand, fig. 6 hardly does justice to this intimacy since the ventral diverticulum of the third pharyngeal pouch conceals the point of diversion of the third and fourth arches. The origin of the fifth arch, however, is definitely located when the sections (figs. 1 to 5) are consulted.

Fifteen microns lateral to the plane containing the point of diversion of the third and fourth arches, the most median and ventral indication of the fifth vessel is seen as a low ridge[2] (fig. 5) on the ventro-caudal surface of the ventral aorta.[3] The ridge increases in size and follows for a very short distance the course of the ventral aorta laterally and dorsally. Continuous with this ridge the fifth vessel has its origin. It curves freely in a latero-dorsal and at the same time, caudal direction (fig. 7), passing between the glandule thyroidienne and the 'prepulmonic caecum.'[4] From this point, the fifth vessel curves rather abruptly towards the median line. Just ventral to its union with the aortic root, the vessel increases greatly in size; this increase is more prominent on the posterior surface (figs. 1 and 7). Opposite this prominence[5] is a slight outbulging on the anterior face of the pulmonic arch. The mesenchyme is continuous between the two vessels (fig. 1). The dorsal lumen of the fifth vessel is situated about midway between the systemic and pulmonic arches, (figs. 1 and 7).

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[2] After the completion of the reconstruction another small island of mesodern was found in this ridge, but is not shown in Fig. 6.

[3] The common trunk of the fourth and fifth arches lateral to the diversion of the third and fourth arches may still be considered as ventral aorta for the following reasons: (1) the distance is only 15 microns; (2) for the same reason that the common trunk of the third and fourth arches isalways regarded as ventral aorta; (3) greater intimacy of the arches is allowable at their points of origin than at their places of termination since they must all arise from a common centre, necessitating the existence of common trunks. But in no instances do the more stable arches normally enter the dorsal aorta by common trunks.

The left side of the same embryo (fig. 10) has only the rudiments of a fifth arch. The vessel seems to have lost its original and most ventral connection, and has formed a second one higher up on the fourth arch. The portion between this and the original connection has partly degenerated. There are dorsal connections with the adjoining arches somewhat similar to those in fig. 8. (In both instances the pulmonic connection is the smaller.) These dorsal branches have persisted and probably represent the connecting sinus found by Tandler in the rat. I have found dorsal connections between the fourth and pulmonic arches in about thirty-five instances in which the dorsal connection varies from a very slender to a broad connection where the fourth and pulmonic arches apparently come in contact (fig. 14). The very slender connections seldom have direct connection with the dorsal aorta.

The 9 mm. sagittal series Number 1299 of the University of Chicago collection is of much interest because of a complete fifth vessel (fig. 8). Twenty microns lateral to the plane of diversion of the third and fourth arches, there is seen on the ventro-caudal surface of the ventral aorta (somewhat similar in position to the vessel on the right side of the preceding embryo) a prominent ridge. After coursing dorsally and laterally 30μ from its most median indication, this ridge abruptly gives off, continuous with its extremity a fifth vessel which, after a very short ventro-caudal course makes a right angle turn in a dorso-caudal direction, curving at the same time laterally. After passing between the much attenuated glandule thyroidienne and the broad 'pre-pulmonic caecum, the vessel curves in a dorso-median direction and joins the dorsal aorta not far from the dorsal lumen of the fourth arch. But just before uniting with the dorsal aorta, the fifth vessel gives off two branches neither of which has so great a diameter as that of the fifth vessel at this point. The smaller of these two branches passes in a caudal direction and joins the pulmonic arch near its dorsal lumen. The larger branch lies practically opposite the branch just described; it passes anteriorly to join the systemic arch just ventral to the union of that vessel with the aortic root.

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[4] For the significance of this term, see part 2.

[5] On the postero-dorsal surface of the systemic arch is also a small prominence of about the same size. These probably indicate the beginning of branches of the fifth arch.

The conditions of this embryo are suggestive of those seen in Lehmann's fig. 12. Their points in common are: that in each case the fifth arch has a branch communicating with the pulmonic arch; these communications leave (or perhaps enter) the fifth arch at about the same angle; the anterior portion of each pulmonic connection is relatively small, while the posterior portion of each is large and flaring. The last fact may be suggested as indicating the connection to have originated from the pulmonic arch. My figure differs from that of Lehmann, in that the fifth vessel of my fig. 8 is more intimately connected with the ventral aorta; the portion entering the aortic root is much larger than the pulmonic branch. These conditions indicate that Lehmann was correct in interpreting the pulmonic connection in her fig. 12 as a branch of the fifth arch. My fig. 9 is made from a reconstruction of a 9 mm. embryo. The rudiment of the fifth vessel has a systemic connection, but is in no way connected with the pulmonic arch. These conditions also point to the correctness of Lehmann's conclusions.

Stages later than 9 mm. exhibit irregularities in abundance, but they gradually become less typical. In a 10 mm. embryo, a pulmonic arch was seen as a Y-shaped vessel. A long fingerlike projection extended down *behind* it from the dorsal aorta. Blunt protuberances were found similarly located in three instances.

Figs. 11, 12 and 13 show irregularities slightly suggestive of a fifth arch, but they are situated so far dorsally and laterally that they need not be considered seriously. Also, they form such straight and direct communications, lacking the curvature found in the typical arches, that I consider it mere conjecture to suggest their having significance. Granting with Locy that irregularities such as these serve to demonstrate the extreme variability of the supposed vessel, it must be admitted that these, and those to which he had reference cannot at the same time prove a typical existence for the new arch.

On the grounds of majority it has been held that a perfect fifth arch has, for its distal termination, the dorsal extremity of the pulmonic arch. It was this consideration that led Locy to oppose the view of Lehmann (above stated and confirmed). But it is evident that, in the pig at least, the fifth arch connects the two aortae and lies between successive pharyngeal evaginations; hence it conforms to the requisites of a typical arch. It may be that conditions other than these represent the highest degree of development attained to by certain forms, but such forms can not be said to exhibit it in a theoretically typical manner. On the other hand, it is only to be expected that the vessel in question generally be found in an a-typical condition and very exceptionally perfect. Therefore an occasional constancy in atypical conditions should not be allowed to dominate our conception of a theoretically perfect arch.

The relatively late appearance of the supposed fifth arch is another point which believers in that vessel have seen fit to neglect. This point receives consideration in Part 2.

PART 2

The double pharyngeal out-pocketing found back of the systemic arch is not well understood. At present there are three views concerning its significance: (1) that the double outpocketing represents the fourth pharyngeal pouch with its dorsal and ventral diverticula (Fox, Tandler, Coulter); (2) that the dorsal and ventral parts of the outpocketing represent the fourth and fifth pouches respectively (Greil, Zimmermann); (3) that the ventral portion represents the postbranchial body of lower vertebrates (Mauer, Verdun). From these considerations the ventral portion of the double outpocketing has been designated by the following terms: (1) ventral diverticulum of the fourth evagination; (2) fifth branchial pouch or ultimo-branchial body; (3) post-branchial body. Since no one of these terms is sufficiently general to cover all the views presented, the term 'pre-pulmonic caecum' might be suggested. This term is applicable to the ventral division of the out-pocketing, whatever may be its significance. The further usefulness of the term is shown later.

This much, however, is definitely settled: the dorsal portion of the double pouch is the anlage of the 'glandule thyroidienne' of Prenant, while the 'pre-pulmonic caecum' becomes the rudimentary lateral thyroid.

According to Verdun the development of the last pharyngeal evagination (or evaginations) is as follows: the pre-pulmonic caecum is first given off as a latero-ventral diverticulum, posterior and mesial to the third pouch. Near the dorsal extremity of the caecum, and slightly anterior to it the "glandule thyroidienne" is given off immediately from the pharynx as an independent diverticulum. Both push out laterally, drawing with them the wall of the pharynx lying between their bases. Thus they acquire secondarily a common pharyngeal orifice. Their formation differs in this last respect from that of the pouches anterior.

Fox ('08) in describing the posterior evagination of a 6.5 mm. pig, gave the location of the 'dorsal portion of the fourth pouch' as posterior and dorsal; he gave the 'ventral portion' as anterior and ventral. This may have been due to his considering directions with reference to the general contour of the embryo, and not with reference to the direction of the pharynx where the evagination joins it.

In the rabbit and the pig the glandule thyroidienne is generally considered anterior to the pre-pulmonic caecum.

The posterior evagination in the cat is figured by Coulter as having dorsal and ventral portions, the dorsal portion having become constricted into anterior and posterior divisions. The anterior division is more directly continuous with the ventral portion, and is labeled the dorsal diverticulum of the fourth pouch. The posterior division is represented as the fifth pouch. If the entire dorsal portion of the evagination be considered anterior, as in other forms, the fifth pouch of the cat would be morphologically anterior to the ventral diverticulum of the fourth pouch. If as stated by Verdun (and as I have observed in a limited study of the development of the evagination in the pig) the earliest indication of the Y-shaped pouch is its ventral portion, Coulter erred when he labeled the most caudal pharyngeal prominence of his 4.5 mm. cat as 'pouches 4-5.'

The 'glandule thyroidienne,' like the pouches anterior to it, comes in contact with the ectodern, differing radically in this respect from the prepulmonic caecum. Occasionally I have found it constricted into dorsal and ventral portions. In one instance (fig. 16) which is very unusual, the glandule thyroidienne is divided into dorsal and ventral portions, each of which has direct connection with the exterior through the pre-cervical sinus. Between the small diverging tubes thus formed, is the mesodermal connection presumably between the fourth and fifth visceral arches. The plane passing through the pre-pulmonic caecum is shown slightly above (posterior to) that of the glandule thyroidienne. The occasional division of the glandule thyroidienne into dorsal and ventral portions is suggestive of its representing an independent pouch.

Attention may profitably be called to the chronological relations of the portions of the double evagination to the associated aortic arches, since these relations have hitherto remained unnoticed. The 'glandule thyroidienne' and the 'fifth' arch, despite their *anterior* position, appear respectively *later* than the pre-pulmonic caecum and the pulmonic arch. (The same conditions are found in birds.)

The dorsal portion of the Y-shaped evagination would appear, then, to correspond to the transient fifth vessel, and to have lost its double nature proportionately to the degeneration of the fifth arch. The tendency towards a distinctness of division of the 'glandule thyroidienne' from the pre-pulmonic caecum closely parallels the tendency towards the development of a perfect 'fifth' arch.

The pre-pulmonic caecum, therefore, is branchial in nature to the extent to which the pulmonic vessel is a true aortic arch; what this extent is, must be determined by a more thorough study than has yet been given to the question. Both seem to have been greatly modified, if they have ever resembled closely the parts anterior which have generally been considered their homologs.

From the foregoing considerations two views are possible: (1) that the new arches so far exploited are merely irregularities which happened to be suggestive of a fifth arch but have no significance; and that the Y-shaped evagination is simply a fourth pouch; (2) that the fifth arch occasionally exists and the intimately related pharyngeal evagination represents more than a fourth pouch. There is much to favor the latter alternative.

CONCLUSIONS

1. The vascular irregularities are more or less common throughout the branchial circulation and may in any part show a slight tendency towards bi-lateral duplication.
2. Even though this tendency is far more pronounced in the region of the supposed fifth arch, the irregularities here should be considered very reservedly, since in most instances they are merely an expression of the tendency towards anastomosis, common to vessels in close juxtaposition.
3. A fifth vessel, very closely approximating a theoretically perfect aortic arch can be demonstrated for the pig; it may be with or without connecting branches from either the systemic or the pulmonic arch or from both.

4. If a typical 'fifth' arch actually exists, appearing after the pulmonic arches are formed the latter vessels must be regarded as differing, to a certain extent, from the vessels anterior to them; and to this extent the branchial nature of the pre-pulmonic caecum has become modified.

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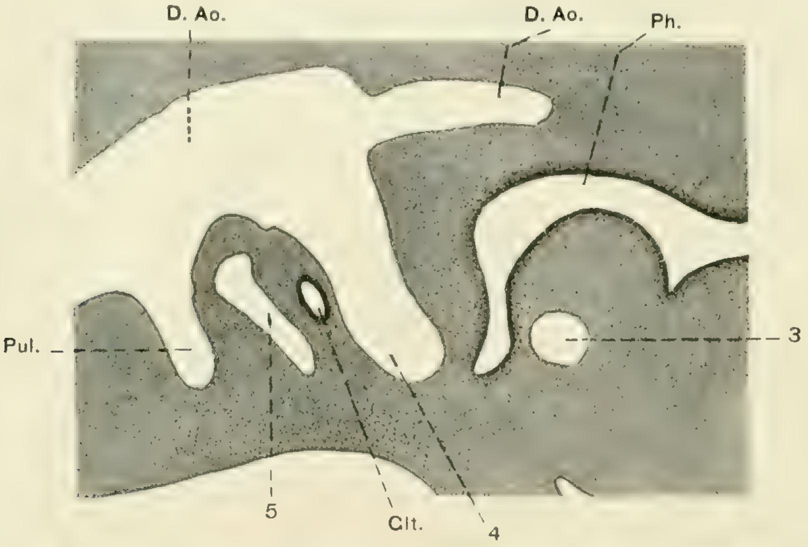
ABBREVIATIONS

D.Ao., Dorsal aorta
Gl.t., Glandule thyroidienne
Mes.c., Mesodermal connection
Ph., Pharynx
Ph. 1., Common pharyngeal lumen of glandule thyroidienne and the prepulmonic caecum
Pul., Pulmonic arch
Sin.pr., Sinus preacervicalis
V.Ao., Ventral aorta
Vd. 3., Ventral diverticulum of third pouch
3, 4, 5., Third, fourth and fifth aortic arches



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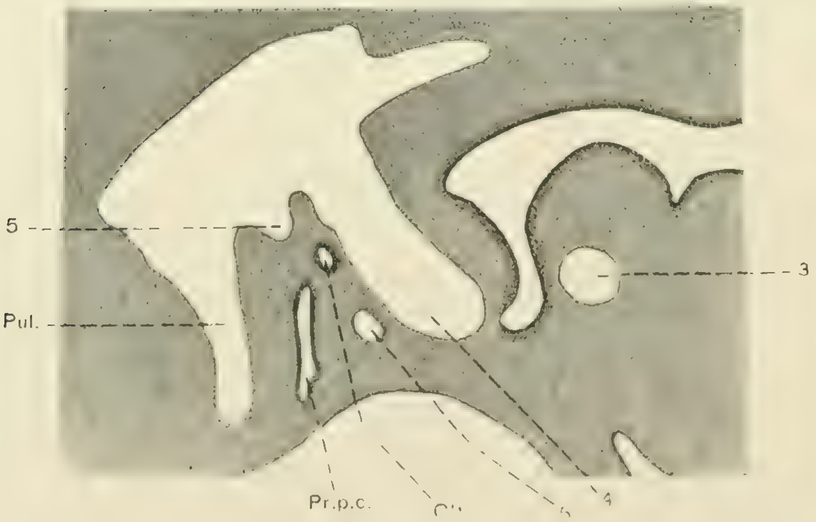

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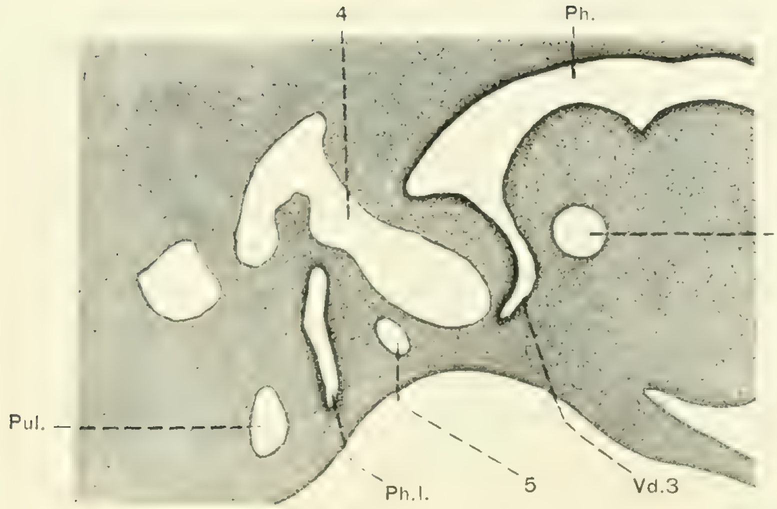
Figs. 1 to 5 Sagittal sections through the branchial region of the right side of the 8.6 mm. pig, series no. 78.8 of the C. H. Spurgeon collection. × 60.

Fig. 1 Section through the upper portion of the fifth arch. The section passes through the glandule thyroidienne about 20μ median to its most lateral extremity.

Fig. 2 Section (40μ median to section 1) through the dorsal lumen of the fifth, and passing through it not far from its middle point. (The prominence on the dorsal portion of the systemic arch is still noticeable.)



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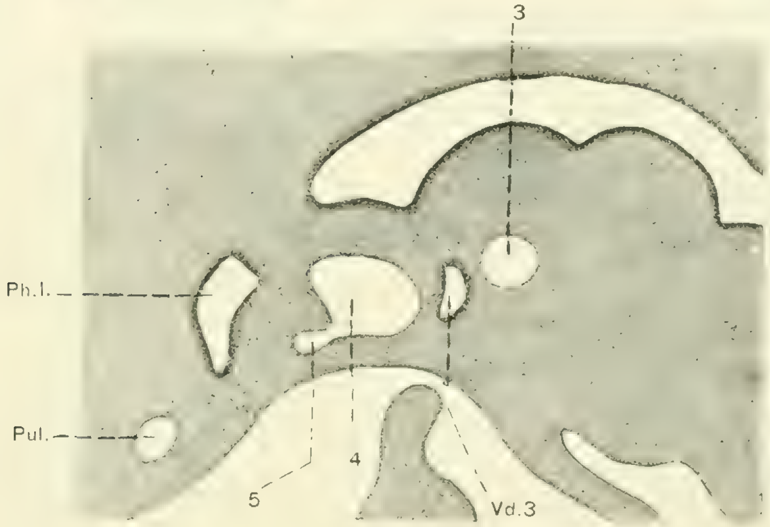

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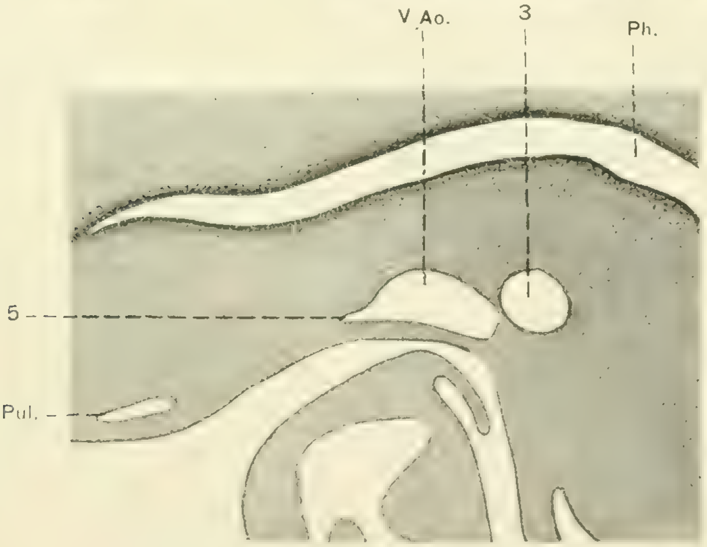

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Fig. 3 Section (20μ median to section 2) through the lower part of the fifth arch, showing the common orifice of the glandule thyroidienne and the prepulmonic caecum.

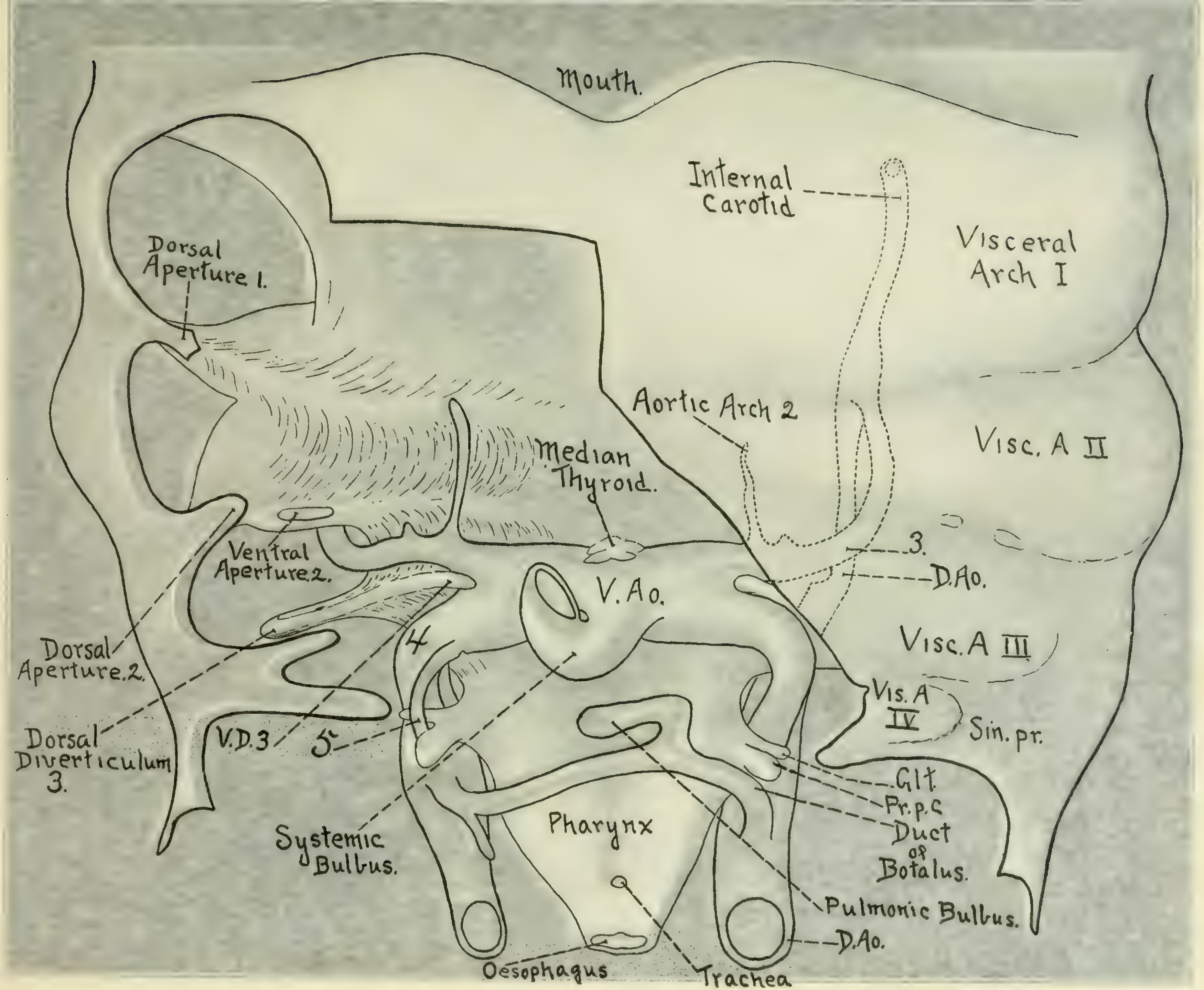
Fig. 4 Section (40μ median to section 3) through the ventral lumen of the fifth arch.

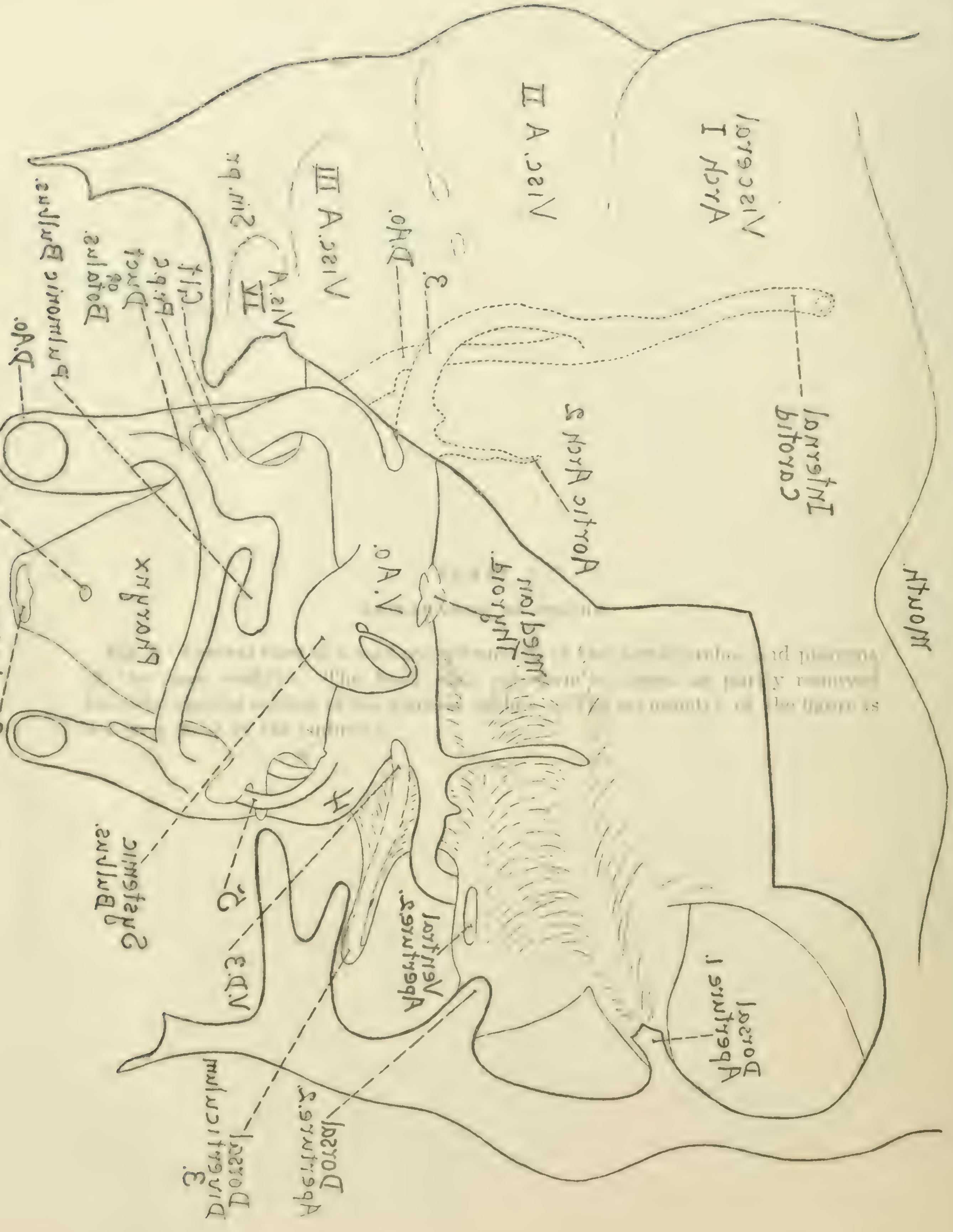
Fig. 5 Section 20μ lateral to the point of diversion of the third and fourth arches, showing the fifth arch about 5 from its most median indication.

PLATE 1

EXPLANATION OF FIGURE

Fig. 6 Ventral view of a wax reconstruction of the aortic arches and pharynx of the same embryo. The body wall ectoderm is shown as partly removed from the ventral surface of the visceral arches. (The asymmetry of the figure is due to a twist in the embryo).


Mouth.
Internal Carotid
Visceral Arch I
Dorsal Aperture 1.
Aortic Arch 2
Visc. A II
Median Thyroid.
3.
D.Ao.
Ventral Aperture 2.
V. Ao.
Visc. A III
Dorsal Aperture. 2.
4
Vis. A IV
Sin. pr.
Dorsal Diverticulum 3.
V.D.3
5
Glt
Pr.p.c
Duct of Botalus.
Systemic Bulbus.
Pharynx
Pulmonic Bulbus.
D.Ao.
Oesophagus
Trachea


Visceral Arch I
Visc. A II
Visc. A III
Vis. A IV
Sin. pr.
D. Ao.
3
Internal Carotid
Aortic Arch 2
Median Thyroid
V. Ao.
Clft
Bpc
Duct of Botalus
Pulmonic Bulbus.
D. Ao.
Trachea
Pharynx
Oesophagus
Systemic Bulbus.
2
V.D. 3
Ventral Aperture 2.
Dorsal Diverticulum 3
Dorsal Aperture 2.
Dorsal Aperture 1.
Mouth

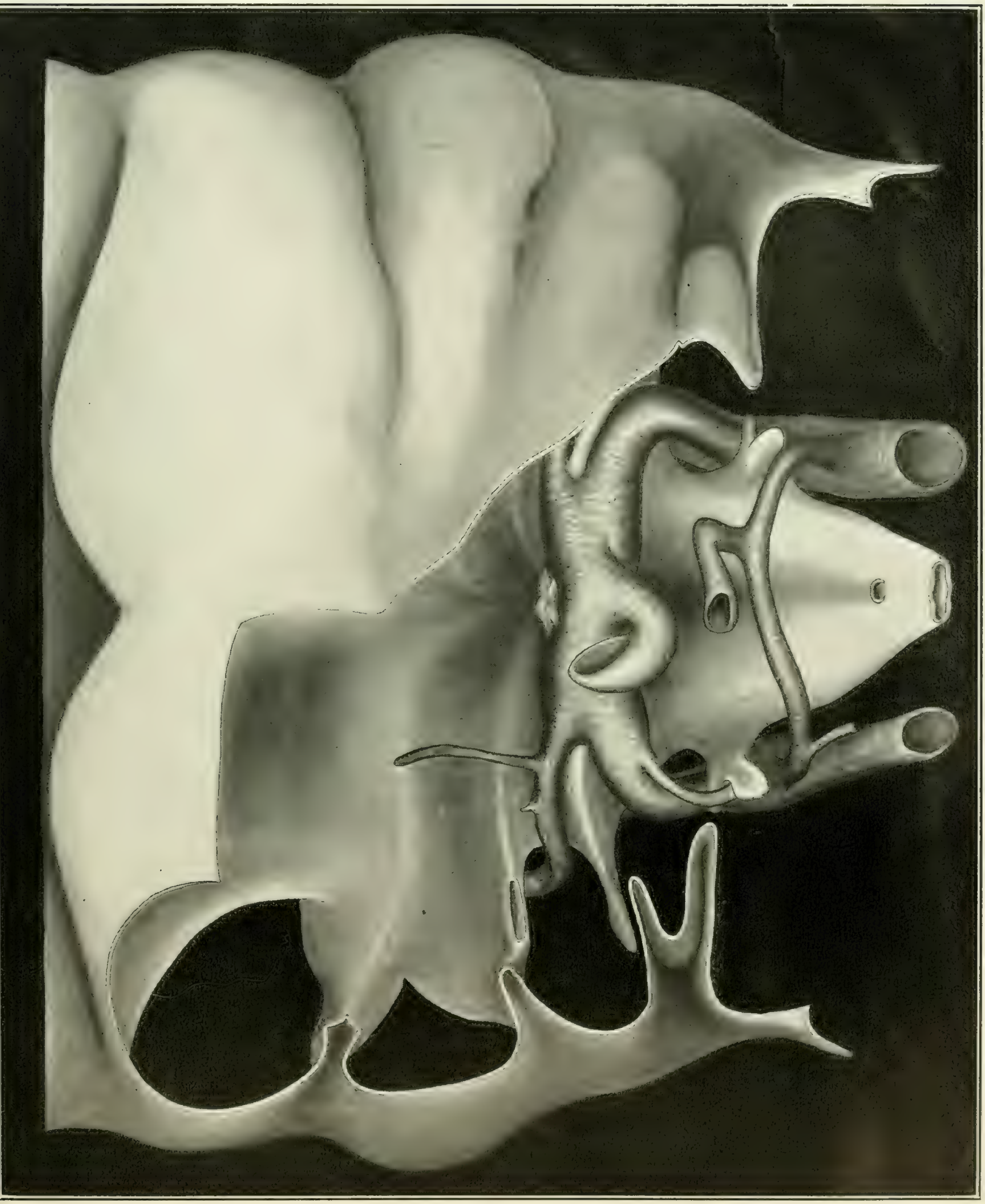
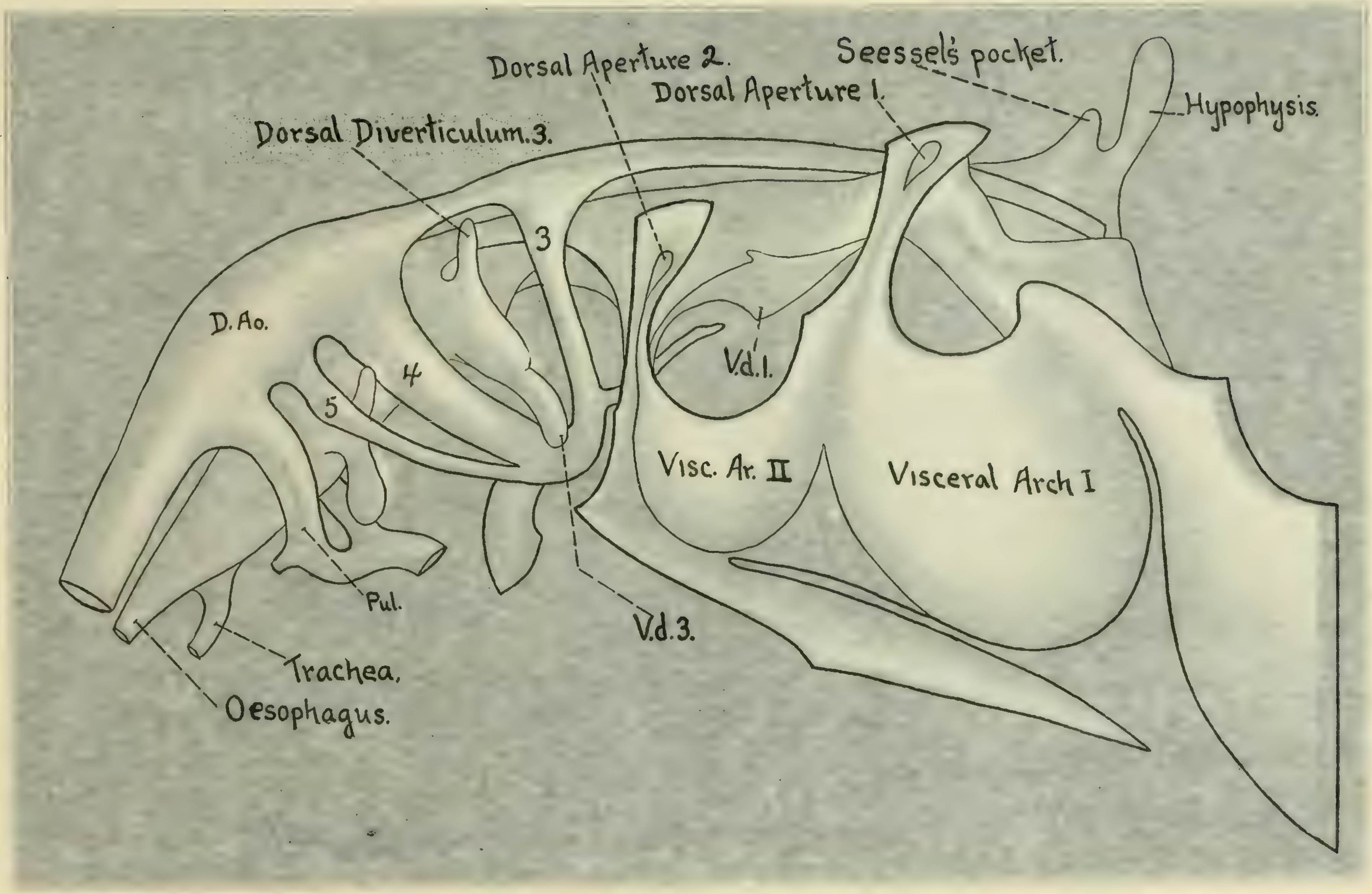
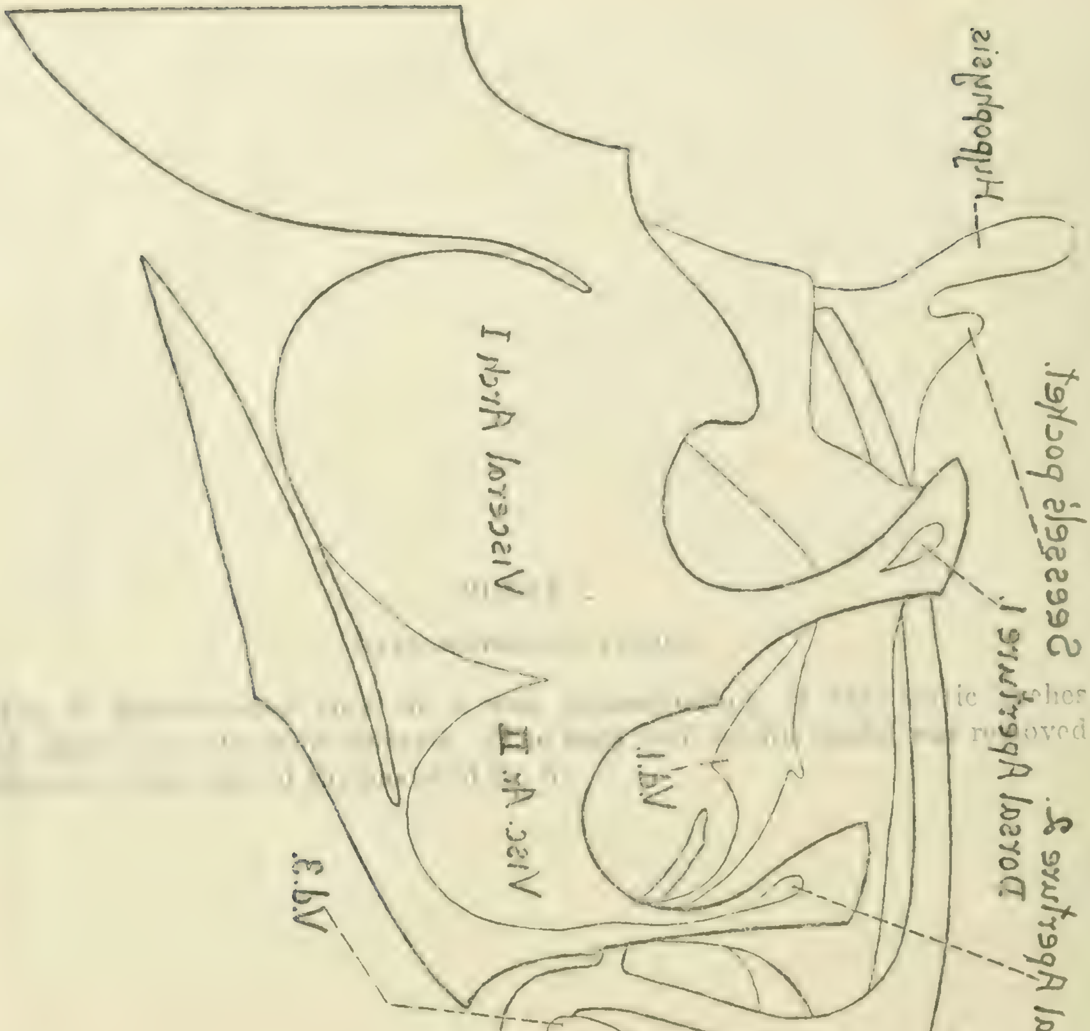


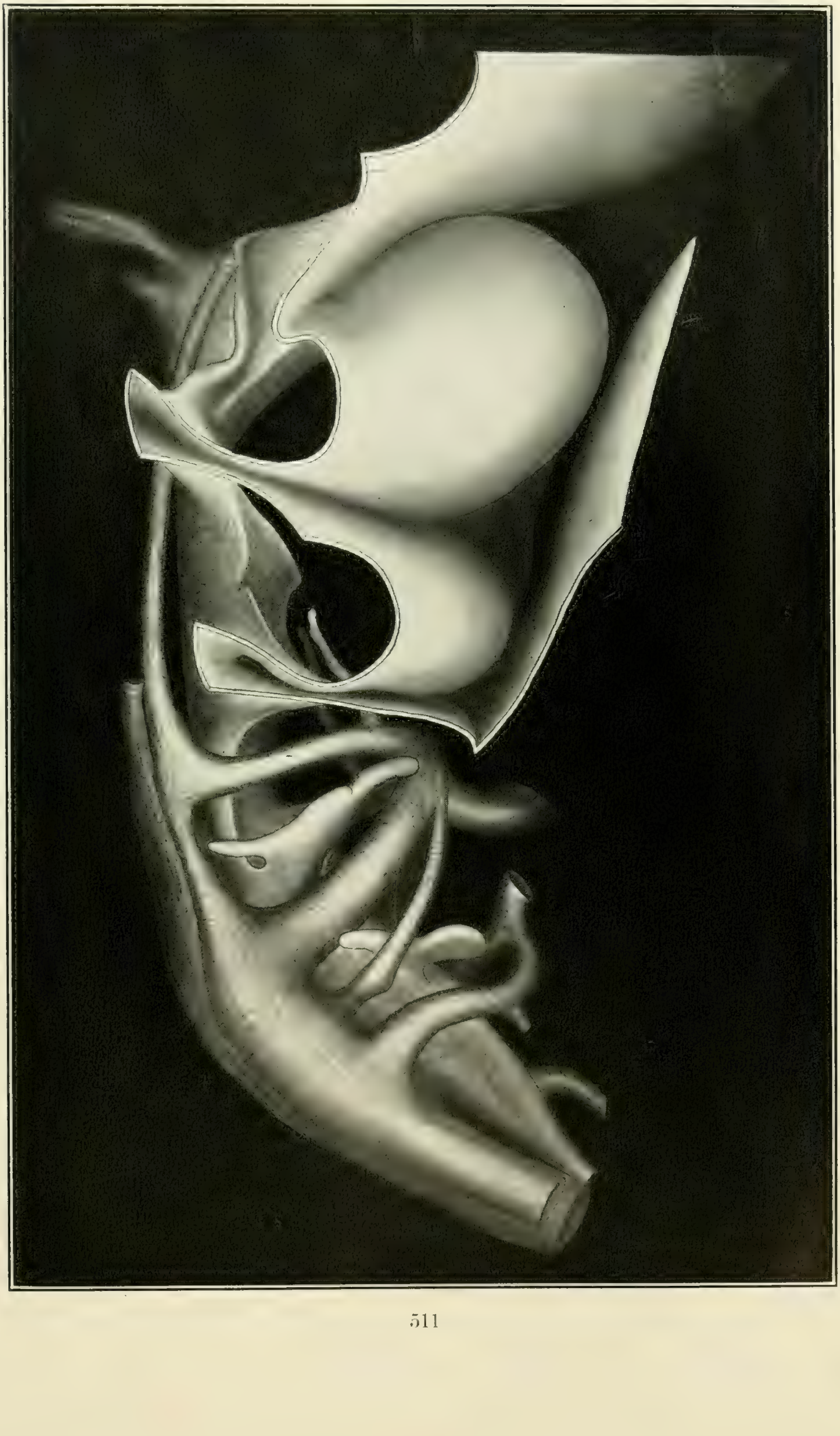
PLATE 2

EXPLANATION OF FIGURE

Fig. 7 Right-lateral view of a wax reconstruction of the aortic arches and pharynx of the same embryo. (The body wall in this model was removed differently from that of the model of fig. 6).


Dorsal Aperture 2.
Seessel's pocket.
Dorsal Aperture 1.
Hypophysis.
Dorsal Diverticulum. 3.
3
D. Ao.
4
5
V.d. 1.
Visc. Ar. II
Visceral Arch I
Pul.
V.d. 3.
Trachea.
Oesophagus.


Hypophysis
Seessels pocket
Dorsal Aperture 1
Dorsal Aperture 2
Dorsal Diverticulum 3
Visceral Arch I
Visc. Ar. II
V.d.1
V.d.3
3
4
5
D.Ao
P.t.
Trachea
Oesophagus



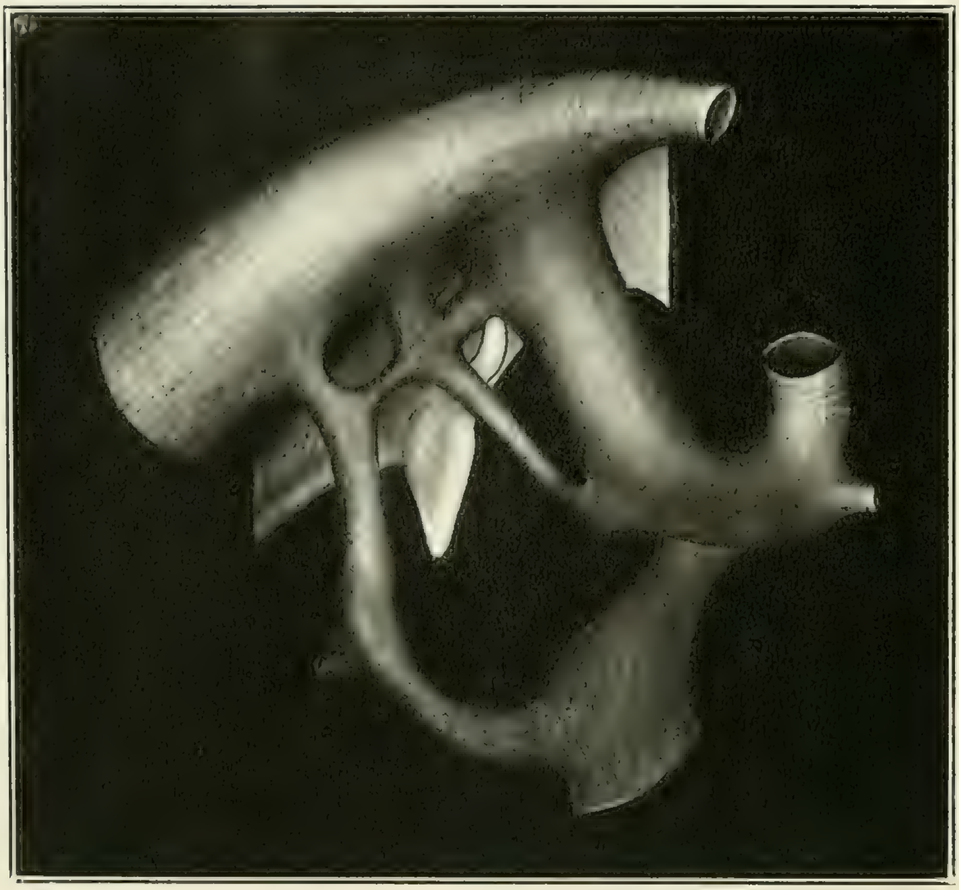
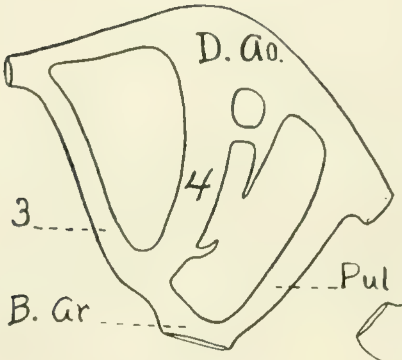


Fig. 8 Reconstruction of the aortic arches of the right side of a 9 mm. pig Series no. 1299, University of Chicago collection.



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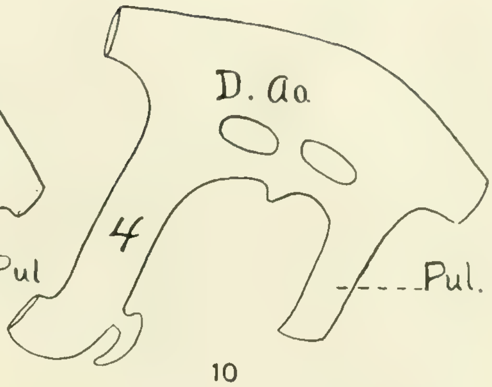

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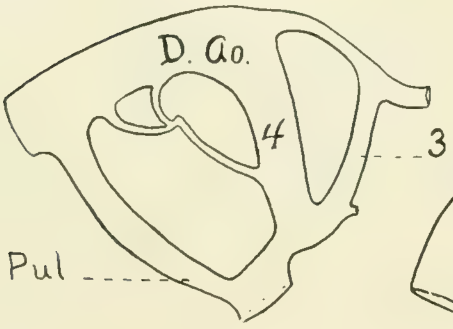

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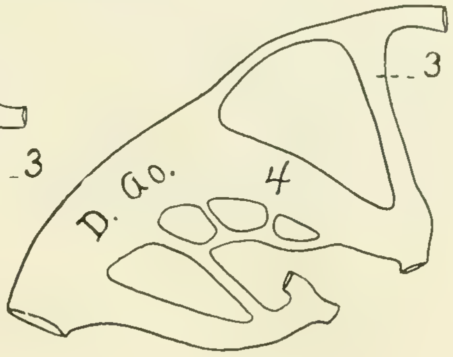

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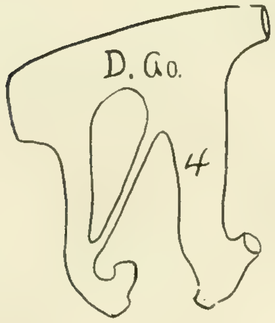

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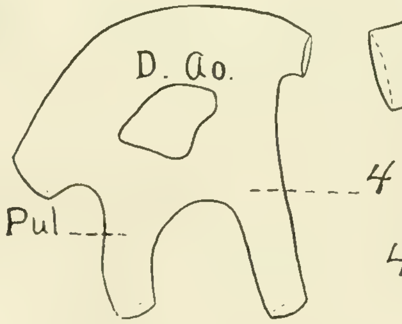

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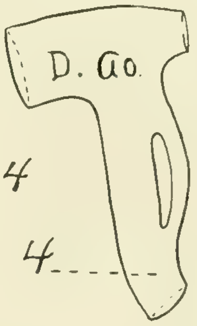

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Fig. 9 The aortic arches of the left side of an 8.5 mm. pig. (The development of the rudimentary vessel seems to have been impeded by the common base of the glandulethyroidienne and the pre-pulmonic caecum).

Fig. 10 The left fourth and pulmonic arches of the 8.6 mm. pig. No. 78.S of the C. H. Spurgeon collection. (No attempt was made to illustrate these conditions in figs. 6 and 7.)

Fig. 11 Aortic arches of the right side of a 9.5 mm. pig, showing irregular conditions.

Fig. 12 Aortic arches of the right side of a 10 mm. pig, showing unusual conditions.

Fig. 13 The fourth and pulmonic arches of the right side of a 10 mm. pig, showing the connection between the base of the pulmonic and the dorsal part of the fourth. (Conditions similar to this were observed in three instances).

Fig. 14. The fourth and pulmonic arches of a 9.6 mm. pig, showing a broad connection between the dorsal portions.

Fig. 15 The fourth arch of a 6.5 mm. pig, showing a division of that vessel on its anterior face.

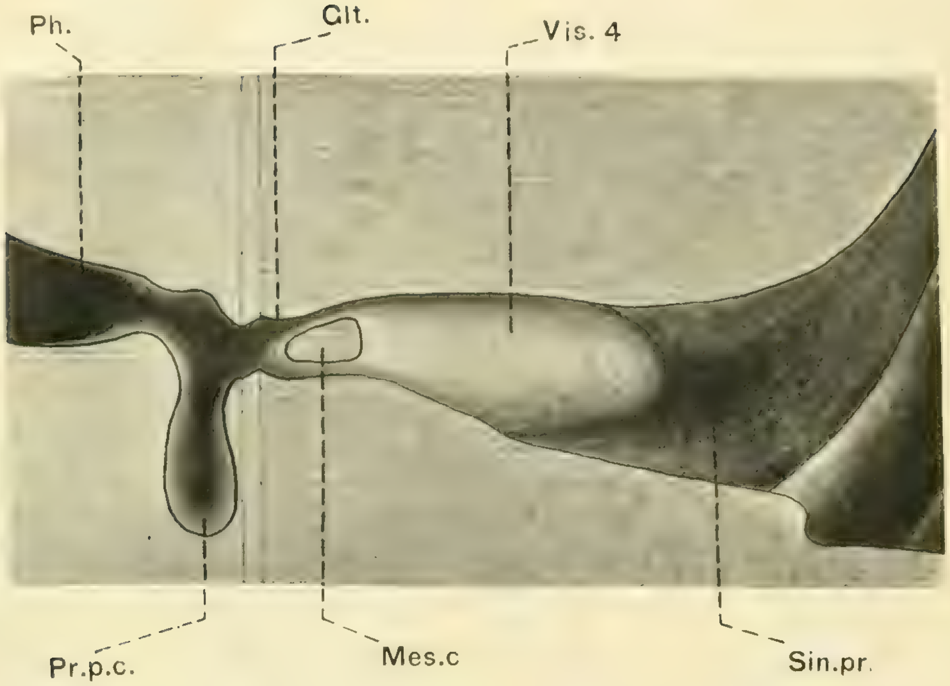

Fig. 16 Reconstruction of the glandule thyroidienne and the pre-pulmonic caecum of an 8 mm. pig. The planes of the cut surfaces (represented as striated areas in the figure) are practically frontal. The lower (i.e., more anterior) plane passes through the glandule thyroidienne. The upper (posterior) plane passes through the pre-pulmonic caecum. The figure is slightly diagramatic.

ADDENDUM

I regret very much to say that the excellent work of Reinke (E. E. Reinke, Anatomical Record, December 1910) had entirely escaped my notice until the present work was sent to press. On the whole, his results agree very closely with my own. The fifth arch of his 6 mm. pig seems more degenerate than that of my figures 1 to 7. It may be questionable that the vessel had existed at all more typically than at the stage at which the embryo was killed. Its ventral origin is perhaps from a more dorsal point; from his fig. 2, the calibre of the fifth arch seems relatively smaller, and its contour is less regular. The conditions in general, support admirably my contention that the fifth vessel does not essentially have the dorsal extremity of the pulmonic arch for its distal termination, since the connection with the aortic root is much the larger. The double nature of the glandule thyroidienne of Reinke's fig. 1 (labeled Pouch Four) strongly suggests its comparability to the anterior pouches. In a few instances I have observed the presence of five ectodermal grooves, but the parallelism between these and the presence of a fifth vessel does not seem constant.

Even with an additional consideration of this valuable contribution, I still believe the existence of a new arch to be somewhat questionable, although there is much evidence in favor of it.

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Anal.
Sept. 25, 1915